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TRANSACTIONS OF THE SOCIETY.

I.—TWO USES OF URANYL NITRATE.

By P. E. HUGHESDON.

(From the Department of Morbid Anatomy, University College Hospital
Medical School, London.)

1. PERMANENT METACHROMATIC STAINING OF MUCIN.

DURING an attempt to elucidate the difficulty of counterstaining reticulum
preparations it was noticed that sections placed in uranyl nitrate and sub-
sequently in methylene blue gave a vivid alcohol-fast metachromatic staining
of mucin. The following method is satisfactory for paraffin sections after
10 p.c. formalin fixation :

1. Hydrate to tap-water.
2. 1 p.c. potassium permanganate 1–5 minutes. Rinse in tap-water.
3. 5 p.c. oxalic acid till colourless. Rinse in tap-water.
4. Stain 2 minutes in 1 p.c. azure B, 0·2 p.c. azure A, 1 p.c. azure C, or
   1 p.c. well-ripened toluidine blue. Rinse in tap-water.
5. Rinse in 0·2 p.c. uranyl nitrate for 10 seconds or longer till sufficient dye
   is extracted to give a good colour contrast. Rinse in tap-water.
6. Blot. Dehydrate in absolute alcohol, clear in xylene, mount in D.P.X.
   medium.

The initial oxidation ensures a fast stain and for most purposes may be of
any duration within the given limits. It should not exceed 1 minute for the
more attenuated types of stromal mucin, as in young connective tissue, as
these substances are very sensitive to permanganate, whilst their relatively

great affinity for thiazin dyes (Hempelmann, 1940) renders fastness no great problem. For the slighter collections of epithelial mucin, on the other hand, as in some stomachs and pseudomucinous cystadenomata, a full 5 minutes is desirable for adequate depth and metachromasia. If in any case a presumptive mucinous area fails to hold the dye and is left unstained, the section should be destained in acid alcohol and the whole sequence repeated *de novo* when the extra permanganate oxidation should induce the required fastness. In no case should the section be returned merely to stage 4, as the sequence uranyl nitrate–dye–uranyl nitrate gives a diffuse metachromatic stain, particularly of collagen.

Results : Mucin, mast cell granules, elastic fibres are crimson or red-violet, other tissue elements various shades of blue.

Comparison with mucicarmine over a wide range of tissues showed general agreement, and suggested that the method detailed is slightly less sensitive than mucicarmine for epithelial mucin and distinctly more sensitive for stromal mucin. The latter conclusion accords with that of Sylvén (1938), who used direct toluidine blue staining.

Rationale.—(1) Stages 2–3. The use of permanganate to increase the intensity and permanence of staining with basic dyes is due to Henneguy (1891). It appears to augment the uptake of cations (basic dyes, iron, aluminium) and to diminish that of anions (acid dyes, ferrocyanide, chromic acid). Alternatives tried were fortification of the dye with phenol, resorcinol, formalin, aniline, and borax, but these gave uneven results with less augmentation of fastness than of initial intensity. Azure A alone is fast enough to be used without preliminaries, but gives a clearer picture, even of stromal mucin, after permanganate despite the latter's destructive action. Epithelial mucin differs specifically in this respect, for though long oxidation, e.g. 1–2 hours, destroys its metachromatic power, together with its affinity for mucicarmine and aniline blue, short periods, up to 15 minutes, enhance it. This is probably due to increased uptake of dye at the required point. It has been shown by Michaelis and Granick (1945) that agar and toluidine blue form a metachromatic compound of inconstant composition and shade, becoming progressively redder with increased adsorption of dye to give larger molecular aggregates. In sections with only faint traces of epithelial mucin a certain minimal intensity of staining is needed before metachromasia can be induced, and to this may be attributed the greater sensitivity of methods, such as mucicarmine, which register only the basophilia of mucin and depend for selectivity on another principle than metachromasia.

(2) Stage 4. Freshly prepared 1 p.c. methylene blue gave a vivid red/blue contrast when wet, but reverted to a variable degree in alcohol and xylene towards a blue-violet/blue-green which is optically unsatisfactory though the wavelength shift is probably not less. The tables of Conn (1946) suggest that the greatest optical effect of a given shift will occur if the primary colour is near to pure blue with an absorption peak in the region of 600 mμ. From blue-green to blue is as far as from blue to red, and the normal uselessness of pure methylene blue as a metachromatic stain is not due to the absence of two well-separated bands (Michaelis and Granick, 1945), but to their lying in the former region. Azures B, A, and C all gave a satisfactory contrast. Saturated

aqueous thionin reacted only feebly and gave too mauve a background : 1 p.c. new methylene blue and 1 p.c. solutions of three recently purchased different makes of toluidine blue gave indifferent results for the same reason as methylene blue. Three different makes of toluidine blue known to be several years old all gave satisfactory contrasts. This change of toluidine blue with age is noted by Conn (1946) and is apparently unexplained. The contrast in behaviour shown here was much more striking than in an ordinary wet preparation. Unsuccessful attempts were made to mature the dye artificially by keeping it in the paraffin oven for a month, by boiling it for a few minutes with neutral and acid solutions of permanganate and periodate, by treatment with bromine water, and by ultra-violet irradiation. A slight improvement was effected by keeping a 1 p.c. solution in 1 p.c. sodium carbonate in the paraffin oven for a week, neutralizing thereafter with sulphuric acid. Methylene green 1 p.c. gave a feeble dirty-grey metachromasia, most noticeable with cartilage, unaffected by uranyl nitrate. Of other basic dyes, moderate metachromasia resulted with 1 p.c. brilliant cresyl blue, 0·33 p.c. cresyl violet, and 1 p.c. pyronin Y ; feeble but just perceptible with 2 p.c. neutral red, 1 p.c. safranin, and 0·16 p.c. unboiled Nile blue sulphate ; none with 1 p.c. toluylene blue, 0·66 p.c. rhodamine B, saturated basic nigrosin in 70 p.c. alcohol, 1 p.c. basic fuchsin, 1 p.c. Victoria blue 4R, saturated Victoria blues B and R, 1 p.c. Bismarck brown : 1 p.c. methyl green behaved like methylene green. The metachromatic staining of mucin and amyloid by 1 p.c. crystal violet was neither enhanced nor preserved. Comparison of the structural formulae given for these dyes (Conn, 1946) suggests that an azin, thiazin, oxazin, or xanthene nucleus is required, that is, a central ring completed by an N or preferably S or O atom.

(3) Stage 5. Comparison was made of uranyl chloride, nitrate, sulphate, and acetate. The best concentrations were 0·1–0·25 p.c. The chloride proved too fierce, the acetate unreliable and diffuse, the sulphate similar to the nitrate, leaching out the dye less fiercely but giving a less satisfactory background colour. The exchange is occasionally an advantage.

Substances recommended for use after thiazin dyes for enhancement or preservation of their metachromasia include alum, " iron sulphate," potassium dichromate, potassium ferricyanide, tannic acid (Unna, 1895), potassium ferrocyanide and ammonium molybdate (Bensley, 1934), mercuric chloride, mercuric chloride and potassium dichromate (Hess and Hollander, 1944, 1947). To elucidate the matter further a number of compounds were tested after methylene blue and azure A, and if active with other dyes with the following results.

No increase of metachromasia resulted with 0·25 and 5 p.c. ferric, potash, and chrome alums, beryllium, magnesium, copper, zinc and manganous sulphates, aluminium trichloride, 0·25 and 3 p.c. chromium fluoride, saturated aqueous salicylic, and 0·1 and 1 p.c. *m*- and *p*-hydroxy-benzoic, pyrogallic, tannic, phosphomolybdic, and phosphotungstic acids : 5 p.c. ammonium molybdate produced a feeble diffuse blue-violet colour : 0·01 and 0·1 p.c. potassium permanganate gave only a weak non-alcohol-fast metachromasia, dilute hydrogen peroxide solution none at all, though eventually bleaching the section. Used *before* the dye, ferric alum produces a better contrast not by lightening the metachromatic colour but by delaying the entry of the lighter shades into the

other tissue elements : with Bismarck brown a pure mucin stain results. Unfortunately this manœuvre diminishes the staining of stromal mucin.

Lightening of the metachromatic colour resulted with 0·25 p.c. potassium dichromate, 0·25 p.c. potassium ferricyanide, 1 p.c. potassium ferrocyanide, 5 p.c. mercuric chloride, 0·25 per cent. gallic acid, 0·25 p.c. sulphosalicylic acid. Results with these different compounds had certain common features : (i) they were better without the initial bleach ; (ii) except with mercuric chloride, all were partially alcohol-fast ; (iii) all were abolished by a few minutes in running tap-water ; (iv) they were inclined to be diffuse ; the metachromatic colour appears most readily in mucin, but almost as readily in collagen and intercellular fluid, and quite readily in other tissue elements, to an extent varying with the dye, reagent, tissue, timing, and concentration : by juggling with these and exploiting the partial reversion in alcohol selective results can be engineered ; (v) with exceptions to be noted, positive results were obtained solely with thiazin dyes, and with certain only of these, namely, methylene blue, azures B, A, and C, thionin, and mature toluidine blue, not with new methylene blue, fresh toluidine blue, and methylene green ; the exceptions were : certain additional metachromatic reactions, (a) brilliant cresyl blue with gallic and sulphosalicylic acids, (b) safranin with gallic acid and with dichromate, (c) toluylene blue with ferricyanide, (d) crystal violet with gallic acid (not alcohol-fast) ; and certain omissions ; (e) mature toluidine blue not with mercuric chloride ; (f) only the three azures with ferrocyanide.

The behaviour of mercuric chloride was unusual. Metachromatic results, tending to be diffuse, were obtained readily with a 5 p.c., barely with a 1 p.c., and not at all with a 0·25 p.c. solution, though readily if 1 p.c. sodium chloride were added. The effect was reversed by tap-water in a few seconds and restored by a rinse in acid. Dyes so treated and rinsed in tap-water become partially or wholly insensitive to further mercuric chloride, dichromate, ferrocyanide, and ferricyanide. The inactivating agent is presumably a hydrolytic product. The technique of Hess and Hollander (1947) with sublimate and dichromate probably exploits this inactivating effect to obtain a selective result.

In an attempt to throw some light on the mode of action of these reagents, rough *in vitro* studies were made by taking 5 c.c. of the solution to be tested and dropping in 1 to 5 drops of a 1 p.c. solution of dye or its equivalent. The presence and colour of a precipitate, not always grossly obvious, were determined by pipetting on to a slide and examining microscopically. With uranyl nitrate precipitation occurred most readily with a 0·1 p.c., less so with 0·25 p.c., and least readily with a 1 p.c. solution. Preliminary addition of alkali short of precipitation aided, whilst a drop or two of 5 p.c. sulphuric acid prevented or redissolved, dye precipitates. This suggests that the effective agent is a product of hydrolysis. In accord with this is the fact that a 0·2 p.c. solution of uranyl nitrate mordants tissues, especially collagen, for basic dyes in a manner similar to molybdates, whereas 1 p.c. uranyl nitrate does not. The remaining effective reagents gave precipitates most readily from stronger solutions and these were usually not soluble in acid. With all combinations giving a metachromatic result in sections a metachromatic precipitate invariably formed *in vitro*. This correspondence was particularly striking with

the thiazin dyes, where the contrast between fresh and mature toluidine blue was again shown. With other dyes there was a fair correspondence, but a few metachromatic precipitates were obtained with no equivalent metachromasia in staining. Probably union with the tissue tends to protect the dye. Phosphomolybdic, phosphotungstic, and tannic acids invariably gave normochromatic precipitates with thiazin dyes. The list of Bank and de Jong (1939) of electronegative substances reacting metachromatically and normochromatically with toluidine blue partially corresponds with the foregoing.

(4) Stage 6. The colour changes and runs in balsam. This effect can be imitated by equal parts of domestic turpentine and xylene, oil of cloves, pure cresol, or pure guiacol, but not by cedar-wood oil. It is possibly due to traces of oily phenols. D.P.X. medium is satisfactory.

DISCUSSION.

The *in vitro* results make it unlikely that the agents reviewed ever literally enhance or preserve the metachromasia initiated by union of dye and mucin. More probably a metachromatic dye-agent complex is formed in the tissues. This may well occur more readily with dye attached to mucin, where it is probably adsorbed as a molecular aggregate of varied composition, than with other tissue components, where, at least in the case of nucleic acid, well-defined salt links are formed (Michaelis and Granick, 1945). The reaction with uranyl nitrate, the conditions of which are fairly stringent, is restricted to mucin and is selective. The other reagents have a greater affinity for the dye and readily form metachromatic compounds in other tissues as well.

For studies of the circumstances in which metachromasia occurs and theories of its mechanism the reader is referred to the papers of Holmes (1924, 1926), Kelley and Miller (1935), Lison (1935), Bank and de Jong (1939), Michaelis and Granick (1945), and for the empirical relations between colour and chemical constitution to Watson's (1918) monograph.

Recent work connects the development of a metachromatic colour with aggregation of the dye molecules, but though this may be a necessary accompaniment it does not appear to be a sufficient cause. Some dyes aggregate in solution without producing a metachromatic band (Sheppard and Geddes, 1944), whilst the formation of both blue and red precipitates by thiazin dyes points the same way, namely, to suggest that an intramolecular change must occur as well. A hypothesis as to its nature should relate both to the facts of metachromasia and to those of colour and chemical constitution.

Of the partially valid empirical rules connecting colour and chemical constitution that of Watson (1914, 1918) alone relates to a functional rather than a structural aspect of the molecule and so could be developed to account for metachromasia. Watson's rule states that the possibility of alternative quinonoid configurations in itself confers a deep colour on a compound. The production of a metachromatic colour may therefore be due to partial or total loss of one or more of these possibilities, of which there are four in the thiazin dyes and three in the rosanilins. Modern theories connect quinone formation with the presence of unshared electrons in the auxochrome groups and their

tendency to pass into the ring. They further envisage the molecule as a whole as corresponding not to any one of its possible alternative configurations, quinonoid or otherwise, but as a mesomeric compromise between them, as a corollary to which the ionic charge is not sharply localized but distributed over its various possible sites. If this balanced state corresponds to the primary colour of a compound, its metachromatic colour might relate to any disturbed state in which the various components of the compromise are less equally represented. Such might be caused by : (i) immobilization at one auxochrome group of its unshared electrons by co-ordination with a kationoid reagent, such as the uranyl ion or mercuric chloride, or, conversely, to release of a previously shared pair by loss of a proton and formation of an imino-base (Lison, 1935) ; (ii) any tendency to asymmetrical localization of the ionic charge in an electric field representing the separate or combined effects of (a) mutual repulsion in a molecular aggregate ; (b) suppression of ionization by alkalis, concentrated indifferent salts or non-polar solvents, with consequent closer association of dye cation with its anion ; (c) adsorption at negatively charged groups (Bank and de Jong, 1939). The varied effects of the last may relate to their strength and capacity to repeal or orientate anions associated with the dye and also to their configuration. Thus large multipolar compounds such as tannic acid may tangle up with the dye and connect at several points with little effect on symmetry ; large unipolar compounds of the type especially studied by Lison may connect only at one point, with a marked effect on symmetry whilst sterically hindering further approach to the dye.

2. MALLORY-TYPE STAIN.

The following method gives results that are reliable with formalin fixation and intelligible with other than very thin sections :

1. Hydrate to tap-water.
2. 1 p.c. potassium permanganate 1–5 minutes. Rinse in tap-water.
3. 5 p.c. oxalic acid till colourless. Rinse in tap-water.
4. 1 p.c. uranyl nitrate 3–5 minutes. Rinse well in tap-water.
5. Stain 3–5 minutes in 0·5 p.c. Biebrich scarlet W.S., 0·1 p.c. orange G., 0·2 p.c. glacial acetic acid. Rinse.
6. 5 p.c. phosphotungstic acid 10 minutes. Rinse.
7. Aniline blue W.S. and conc. HCl a.a. 0·25 p.c. q.s., usually 1–6 hours, depending on the tissue and the temperature. Rinse.
8. Blot, dehydrate rapidly in absolute, clear in xylene, mount in balsam.

All rinses from stage 5 onward to be in aq. dist. or acetified tap-water. It is inadvisable to dehydrate in alcohol containing much eosin.

Rationale.—(1) Stages 2–4. It is generally conceded that the best fixative for this type of stain is Zenker and that the worst is aqueous formalin. Formalin-fixed tissue is faulty in at least three respects : (i) it is conspicuously basophilic and so fails to hold the acid plasma stain and readily takes up the counterstain which, though an acid dye, has an affinity for basophilic tissue elements ; (ii) it lacks the trivalent chromic cation which forms fast lakes with

ponceaux and certain other dyes (Thorpe and Linstead, 1946) ; (iii) it probably lacks something effected by chromic oxidation, for the brief permanganate treatment used above sharpens the fibre stain.

The most convenient of the established remedies is to post-chrome, since all three factors are dealt with together. The customary dichromate bath for several hours, however, is tedious, detergent, and of limited efficacy. Chromium may be inserted rapidly by either (*a*) a few minutes in 5 p.c. potassium dichromate and 1 p.c. v/v sulphuric acid, a brief rinse, and a few seconds' reduction in 2 p.c. sodium bisulphite ; or (*b*) a few minutes in 5 p.c. chrome alum if preceded by permanganate. In present sequence uranyl nitrate is substituted for chrome alum : it appears to be more effective and gives a more translucent plasma stain. The pallor of the plasma stain may be enhanced to any desired degree, as e.g. in thick sections and/or where there is much acidophil cytoplasm, by prolonging the initial oxidation, which, however, increases the time needed in the counterstain. The peculiar usefulness of group VIa metals in this type of stain is noteworthy.

(2) Stages 5–7. Neither originality nor special virtues are claimed for this sequence, which is based on some of the information elicited by Lillie (1940, 1945). It is included as it has been found constantly reliable and contains nothing that can be omitted without detriment. The principle can be adapted to other sequences, provided that the orange G be mixed with the plasma and not the fibre stain.

All solutions employed throughout this study were in aq. dist. unless otherwise stated.

All tissue was human, fixed in 10 p.c. formalin and embedded in paraffin.

The makes and colour index numbers of the dyes employed were as follows :

B.D.H.—Toluidine blue (925), cresyl violet, Nile blue (913).

G. Grübler & Co.—Toluidine blue (925).

E. Gurr.—Methylene blue (922), azures B, A, and C, thionin (920), methylene green (924), toluidine blue (925), new methylene blue (927), Victoria blue 4R (690), Victoria blues R (728) and B (729).

G. T. Gurr.—Methylene blue (922), azures B and A, toluidine blue (925), brilliant cresyl blue (877), basic fuchsin (677), methyl green (684), aniline blue W.S. (707), Bismarck brown (331), toluylene blue (820).

Lucius and Brüning.—Nigrosin, spirit soluble (860).

" *Revector.*"—Orange G (27), Biebrich scarlet W.S. (280), toluidine blue (925), neutral red (825), crystal violet (681), pyronine Y (739), rhodamine B (749).

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II.—PEPPER AND PEPPER HUSK.*

By T. E. Wallis, D.Sc. (Lond.), F.R.I.C., and D. K. Santra, Ph.D. (Lond.), M.Pharm. (Benares).

(From the Museum of the Pharmaceutical Society of Great Britain.)

One Text-Figure.

Introduction.

When pepper is prepared for table use by grinding the berries, numerous products result, and the products are related to the distribution of the sclerenchyma in the fruit. The most important products of grinding are: (1) *powdered black pepper*, which consists of the entire fruits powdered ; (2) *powdered white pepper*, consisting of the fruits from which the outermost layers of the pericarp have been removed ; (3) *black pepper shell*, often described as " husk," consisting of the outer part of the pericarp, removed when converting black pepper to white pepper ; (4) *white pepper shell*, consisting of the outer layers of white pepper, removed in the preparation of a high grade of powdered white pepper ; and (5) *high grade white pepper*, consisting chiefly of finely powdered perisperm.

The products (3) and (4), namely, black pepper shell and white pepper shell respectively, are waste products, and since they are parts of the true pepper fruit, they are particularly suited for the adulteration of commercial grades of ground pepper, because it is very difficult to determine the excess amounts of these layers that may be present in the adulterated material. It has been customary to rely upon two methods in general, one based upon the amounts of crude fibre present and the other relying upon a comparison made by the eye of the microscopical appearance of the pepper with adulterated samples of known composition.

Neither of these methods is sufficiently precise to be entirely satisfactory, and prosecutions based upon results obtained by using them have failed to carry conviction in the courts of law. When giving his judgment in the important " White Pepper Case " in 1914 (Sale of Food and Drugs Act), the Recorder stated that the evidence to which he had listened had failed to clarify certain important points which had arisen during the trial. These were :

(1) How much of the pericarp constituted " husk " ?

(2) What proportion of " husk " is normally present in white pepper ?

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* The subject-matter of this communication formed part of a Thesis approved for the degree of Doctor of Philosophy of the University of London.

(3) Crude fibre figures did not appear to provide an accurate estimate of " husk " present in the pepper ; and

(4) Microscopical examination of stone cells in the powdered pepper gave unreliable estimates of " husk."

PURITY OF PEPPER.

Certain constituents of pepper are commonly used as criteria of purity for pepper. They are : crude fibre, non-volatile ether extract, starch, and ash. These are satisfactory to the extent of checking consignments of pepper against samples previously submitted by the merchant, but when pepper has been adulterated with waste products, especially those derived from the pepper fruit, these standards are found ineffectual. For example, black pepper contains 10·85–14·36 p.c. of crude fibre, and black pepper shell contains 21·65–28·52 p.c. of crude fibre (Smith *et al.*, 1926). Now, to a sample of black pepper with a crude fibre of 11 p.c., as much as 25 p.c. of the shell with a crude fibre of 22 p.c. could be added without bringing the resulting crude fibre, calculated to be 13·83 p.c., outside the range of crude fibre values for genuine black pepper.

Again, black pepper contains 7·25–10·74 p.c. of non-volatile ether extract and black pepper shell contains 6·40–9·54 p.c. of the same extract (Smith *et al.*, 1926). Calculation shows that 25 p.c. of shell containing 7·0 p.c. of " non-volatile ether extract," added to black pepper containing 10·0 p.c. of non-volatile ether extract, would not bring the non-volatile ether extract of the mixture, namely, 9·25 p.c., below the minimum found in samples of genuine black pepper. Similarly, the starch and ash contents have been found to be ineffectual in excluding a significant amount of added shell.

Standards based on these contents are also ineffectual in the case of white pepper. For example, the standards suggested by the Joint Committee on Definitions and Standards, Bureau of Chemistry, Washington, D.C. (1917), require that white pepper should contain not less than 7 p.c. of non-volatile ether extract, not less than 52 p.c. of starch, not more than 3 p.c. of total ash, and not more than 5 p.c. of crude fibre. Samples of genuine white pepper contain 1·03–4·86 p.c. of crude fibre (Smith *et al.*, 1926), hence to samples of white pepper containing 2 p.c. of crude fibre, as much as 10 p.c. of black pepper shell and even more of white pepper shell could be added without bringing the crude fibre of the mixture, namely, 4 p.c., outside the maximum standard for genuine white pepper.

MICROSCOPICAL METHODS.

As pointed out above, the microscopical method of comparison and assessment of quantity by eye in the " White Pepper Case " appeared to be unreliable to the judge, because it gave widely different results for the amount of " husk " found in the same sample of pepper examined by different analysts. Since substances (Wallis and Santra, 1947, 1948) containing sclereids or sclerenchymatous cells can be determined quite accurately by the " lycopodium method " (Wallis, 1920) of counting the sclereids, it seemed possible that these

problems could be satisfactorily solved by a study of the sclerenchymatous cells, both the stone-cells of the hypodermis and also the " beaker-cells " of the inner epidermis of the pepper fruit. The following is an account of the work undertaken with this end in view.

One of the problems raised in the " White Pepper Case," referred to in the introduction, was that some confusion existed in the definition of the term " husk."

The accompanying drawings, made from transverse sections of pieces of commercial black pepper shell and of white pepper shell, show the structure of these two waste products. Black pepper shell (see fig. 1, B), usually consists
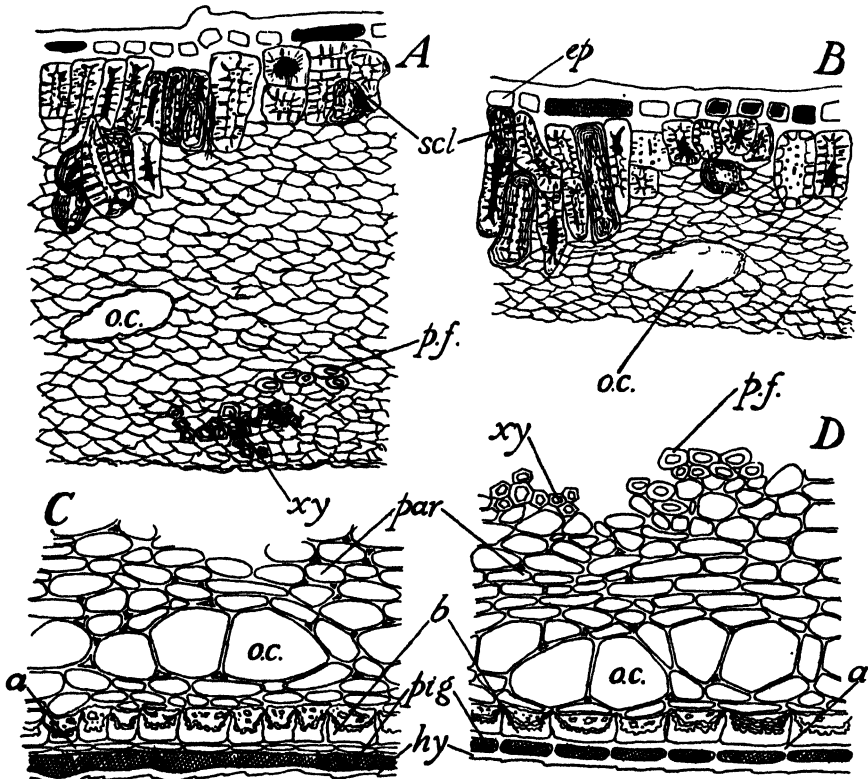
Fig. 1.—Pepper " shells "; transverse sections. A and B black pepper shells; c and D, white pepper shells. *a*, outer epidermis of the testa; *b*, beaker-cell layer (inner epidermis of the pericarp); *ep.*, outer epidermis of the pericarp; *hy.*, hyaline layer; *o.c.*, oil-cell; *par.*, parenchyma; *p.f.*, pericyclic fibres; *pig.*, pigment layer of the testa; *scl.*, sclereids of the hypodermal layer; *xy.*, xylem vessels of a vascular strand. All × 200.

of the epicarp of heavily cuticularized epidermal cells, *ep*, a hypodermal layer with characteristic sclereids, *scl*, with dark contents, and the outer mesocarp of shrunken parenchyma containing scattered oil-cells, *o.c.* Very rarely pieces of black pepper shell include some vascular bundles, *xy*, and pericyclic fibres, *p.f.* (see fig. 1, A). White pepper shell, fig. 1, D, consists of the inner parenchymatous mesocarp, in the outer part of which the vascular bundles are embedded and having a layer of large oil cells in the inner part, together with the inner epidermis of the pericarp, which is developed as a layer of beaker cells, *b*, and the following layers from the seed-coat, namely, the outer epidermis, *a*, the

pigment layer, *pig*, and the hyaline layer, *hy*. Occasional fragments of white pepper shell are devoid of vascular strands, as shown in fig. 1, C.

The following experiments relate to adulteration of pepper with " black pepper shell," commonly referred to as pepper " husk."

Number of sclereids per mg. of black pepper shell.—The presence of black pepper shell in ground white pepper can be established microscopically by the detection of the characteristic sclereids of the hypodermal layer of the pericarp, which differ in appearance and distribution from the sclereids (beaker cells) of white pepper. The proportion of black pepper shell in mixtures could be determined by establishing a standard figure for the sclereids of black pepper shell; the number of sclereids per mg. of black pepper shell was therefore determined by the lycopodium method, associated with the disintegration by nitric acid of the irregular masses of sclereids. The details of the method are as follows.

A sample of genuine black pepper shell was reduced to a No. 85 powder in the laboratory. About 0·2 gm. of this powder was accurately weighed and triturated thoroughly in a glass mortar with sufficient of nitric acid, 50 p.c., to make a thin paste. It was then treated with a few drops of phloroglucin and hydrochloric acid. About 0·05 gm. of lycopodium, accurately weighed, was added and mixed thoroughly. The mixture was then transferred to a corked tube with small portions of a suspending medium consisting of 3 volumes of glycerin and 1 volume of hydrochloric acid. The final volume of the mixture in the corked tube was about 5 ml. Suspension of the materials was then completed by shaking the tube gently for a few minutes. Slides were prepared from this suspension and from another made in the same way. Counts were made of sclereids in seven strips across each cover-glass, as was done for the count of sclereids in clove stalk (Wallis and Santra, 1947). The lycopodium spores were counted in twenty-five fields selected to represent uniformly the area covered by the seven strips. The number of sclereids per mg. of black pepper shell was calculated in the following way, taking the counts on one slide as an example :

Weight of lycopodium = 0·025 g.

Weight of black pepper shell = 0·100 g.

Number of lycopodium spores in twenty-five fields (= 2·403 sq. mm.)=308

Number of sclereids in seven strips across the cover-glass (= 26·04 sq. mm.) = 924

Number of lycopodium spores in the seven strips $= \dfrac{308 \times 26\cdot04}{2\cdot403}$.

Hence $\dfrac{308 \times 26\cdot04}{2\cdot403}$ spores correspond to 924 sclereids and 94,000 spores

(1 mg. of lycopodium) correspond to $\dfrac{94,000 \times 924 \times 2\cdot403}{26\cdot04 \times 308}$ sclereids.

Since the proportion of lycopodium to black pepper shell in the mixture is

$1:4$, therefore 1 mg. of black pepper shell contains $\dfrac{94,000 \times 924 \times 2 \cdot 403}{26 \cdot 04 \times 308 \times 4}$

sclereids = 13,010 sclereids.

Two more slides from this suspension were similarly examined. A second group of three slides was prepared from another suspension containing 0·05 g. of lycopodium and 0·1 g. of black pepper shell.

The results from the six slides were as follows :

			Mean	Grand Mean
Suspension I	Slide A.	13,010		
	Slide B.	13,130	13,213	
	Slide C.	13,500		
				13,231
Suspension II	Slide A.	13,250		
	Slide B.	12,800	13,250	
	Slide C.	13,700		

Expressed in significant figures this result gives 13,230 sclereids, with a variation of ± 20, per mg. of black pepper shell.

Determination of black pepper shell in ground white pepper.—In order to test the possibility of determining the proportion of black pepper shell present in ground white pepper, experiments were made on two different mixtures of powdered white pepper with black pepper shell. Both these powders were of No. 85 fineness and were prepared in the laboratory. The mixtures were made by a fellow research worker and given to one of us (D.K.S.) for analysis. The amounts of black pepper shell present in the mixtures were determined by making counts of the sclereids in slides prepared from a suspension of each mixture. The suspension of each mixture was prepared as described for the count of sclereids in black pepper shell (see above). The following is an example giving complete data for one experiment :

Weight of lycopodium = 0·051 g.

Weight of mixture (1) = 0·2146 g.

Number of lycopodium spores in twenty-five fields (= 2·403 sq. mm.)=160

Number of sclereids in seven strips across cover-glass (=26·04 sq. mm.)= 154

$\therefore$ number of spores in 26·04 sq. mm. $= \dfrac{160 \times 26 \cdot 04}{2 \cdot 403} = 1734$

Since 1734 spores correspond to 154 sclereids, $\therefore$ 94,000 spores (1 mg. lycopodium) correspond to $\dfrac{94,000 \times 154}{1734} = 8345$ sclereids

Since 1 mg. of lycopodium corresponds to $\dfrac{0 \cdot 2146}{0 \cdot 051}$ of the mixture, $\therefore$ 1 mg. of mixture contains $\dfrac{8345 \times 0 \cdot 51}{0 \cdot 2146} = 1984$ sclereids.

Four slides made from this suspension of mixture 1 were examined with the following results :

	No. of spores in 25 fields.	No. of sclereids in 7 strips.	No. of sclereids per mg. of mixture.
Slide A.	160	154	1984 ⎫
Slide B.	147	96	1346 ⎬ 1678
Slide C.	145	118	1677 ⎪
Slide D.	163	135	1707 ⎭

Since pure black pepper shell contains 13,230 sclereids per mg., the proportion of " husk " in the mixture works out at 12·6 p.c. This result of 12·6 p.c. was further corrected because the white pepper used in preparing the mixture contained, as is usually found, a small proportion of black pepper corns as an impurity. From 100 g. of the unground white pepper all black pepper shell was separated from the black pepper corns present and was weighed; it amounted to 0·49 p.c. of the sample. When this amount is deducted the amount of pepper " husk " found in the mixture becomes 12·11 p.c. The amount actually present was 11·9 p.c., so that the percentage error is 1·8.

A second mixture, containing a very small percentage of black pepper shell was next examined and the counts for three slides made from the suspension were as follows :

	No. of spores in 25 fields.	No. of sclereids in 7 strips.	No. of sclereids per mg. of mixture. ·
Slide A.	151	38	545 ⎫
Slide B.	218	52	517 ⎬ 520
Slide C.	204	47	499 ⎭

Using the standard figure of 13,230 sclereids per mg. of black pepper shell, this result of 520 sclereids per mg. corresponds to 3·9 p.c. of black pepper shell and, correcting this for the 0·49 p.c. of shell in the white pepper, the final result is 3·41 p.c. of added black pepper shell. The amount actually present was 3·0 p.c., so that the percentage error is 13·6 p.c.

A point worthy of notice is that so small an amount as 3 p.c. of black pepper shell does not escape determination as it would do by the use of other methods of assay. The percentage error upon such a small quantity of material is necessarily comparatively large, but the result obtained is so close to the actual amount present that the error involved is of no practical importance.

Number of sclereids per mg. of black pepper.—To find the proportion of black pepper shell, or " husk," present in normal black pepper, it is necessary first to determine the number of sclereids per mg. present in the hypodermal tissue of pure black pepper ; this was done by two methods, (*a*) using powdered black pepper without previous treatment, (*b*) using the crude fibre prepared from powdered black pepper.

(*a*) Using powdered black pepper without previous treatment :

A quantity of whole black pepper was freed from foreign matter and was reduced to a No. 85 powder in the laboratory. Counts of sclereids were made by using two suspensions prepared from this powder in the same way as was done for counts of sclereids in black pepper shell; see above. The number of sclereids per mg. of black pepper was calculated from counts of lycopodium spores and of sclereids on slides prepared from these suspensions. The details of the weighings, counts, and calculations are exactly similar to those recorded above for the count of sclereids in black pepper shell. The results for the two suspensions are summarized as follows :

	No. of spores in 2·403 sq. mm.	No. of sclereids in 26·04 sq. mm. (7 strips across the cover-glass).	No. of sclereids per mg. of black pepper.
Suspension I—			
Slide A.	294	288	4248 ⎫
Slide B.	282	338	5196 ⎬ 4557 ⎫
Slide C.	278	271	4228 ⎭ ⎪
			⎬ 4512
Suspension II—			⎪
Slide A.	157	390	5385 ⎫ ⎪
Slide B.	210	375	3870 ⎬ 4468 ⎭
Slide C.	198	379	4150 ⎭

(*b*) Using the crude fibre prepared from powdered black pepper.

The crude fibre was prepared by boiling for $\frac{1}{2}$ hour 20·4 g.m. of the No. 85 powder with 1·25 p.c. sulphuric acid, filtering, washing the residue with distilled water, and boiling it again with 1·25 p.c. caustic soda solution for $\frac{1}{2}$ hour, filtering, washing the residue with distilled water followed by alcohol 90·0 p.c., and finally drying by exposure to the air. The weight of the crude fibre was found to be 4.4 gm. Since the proportion of sclereids in this crude fibre is much larger than that in the original No. 85 powder, a much smaller quantity of it was used for making counts, which were made by the same method as was used for the count of sclereids in the original powder of black pepper. Two suspensions were prepared and from the counts made on slides prepared from them the number of sclereids per mg. of this crude fibre was obtained. The result was corrected to represent the original powder of black pepper.

The counts of spores and of sclereids on all slides and the results derived from them are as follows :

	No. of lycopodium spores in 25 fields.	No. of sclereids in 7 strips across the cover-glass.	No. of sclereids per mg. of black pepper.
Suspension I—			
Slide A.	120	317	4940 ⎫
Slide B.	112	239	4990 ⎬ 4845 ⎫
Slide C.	108	288	3990 ⎪ ⎪
Slide D. ... • ...	112	327	5460 ⎭ ⎪
			⎬ 4650
Suspension II—			⎪
Slide A.	231	550	4454 ⎫ ⎪
Slide B.	210	494	4400 ⎬ 4455 ⎭
Slide C.	187	451	4512 ⎭

The results obtained by these two methods, viz., 4512 and 4650, are in close agreement and the mean gives a final value of 4585 sclereids per mg. of black pepper with an extreme variation of ± 450.

The advantages of making the counts on a crude fibre rather than upon the original powdered black pepper may be summarized as follows :

1. The number of sclereids present in the original powder is so small that an unreasonably large number of fields must be examined. In the crude fibre the sclereids are concentrated and the increase in number greatly simplifies the counting.

2. The large amount of starch present in the original powder reacts with the nitric acid used for disintegrating the sclereid masses, producing numerous bubbles in the microscopical mounts. During the preparation of the crude fibre, however, starch and other cell contents are removed, thus yielding mounts in which the particles are nicely cleared and bubbles are entirely absent.

Proportion of " husk " in black pepper berry.—The proportion of the tissues collectively termed " black pepper shell " in the black pepper berry can now be determined from the sclereid-counts of black pepper shell and of black pepper. Thus :

From the weight of 100 black pepper berries the average weight of one berry was found to be 48 mg.

Since 1 mg. of black pepper contains on an average 4585 sclereids, therefore 48 mg. (i.e. 1 average berry) contains 4585×48 sclereids.

But 1 mg. of black pepper husk contains 13,230 sclereids, therefore amount of husk containing $4,585 \times 48$ sclereids is $\dfrac{4,585 \times 48}{13,230} = 16.63$ mg.

Hence 48 mg. of black pepper contain 16·63 mg. of husk = 34·64 p.c.

This result was verified by carefully scraping the husk from twenty-seven typical berries selected from the same sample of black pepper and weighing the products.

Weight of twenty-seven berries = 1090 mg.

Weight after husk removed = 710 mg.

Therefore, weight of husk by difference = 380 mg. (actual weight of husk collected is 365 mg., therefore loss during manipulation is 15 mg.).

Amount of shell in an average black pepper berry calculated by using the weight of husk obtained by difference is $\dfrac{380 \times 100}{1090} = 34.86$ p.c.

There is a close agreement between the two results obtained by these two independent methods, indicating that the count of sclereids is quite reliable for providing information about the distribution of the tissues containing them. Such counts are especially useful when it is not possible to separate the tissues for direct weighing.

Note.—A study of white pepper shell and its detection in powdered pepper will be made the subject of a future communication.

SUMMARY.

1. The grinding and sifting of pepper for the preparation of ground peppers for dietetic purposes results in the production of two waste products, known respectively as " black pepper shells " and " white pepper shells."

2. Black pepper shells or pepper " husk " has been reported as an adulterant of white pepper. The existing methods for the detection and assay of the " husks " in white pepper are unsatisfactory.

3. The structure of pepper " husks " reveals the presence of a large proportion of sclereids, or stone-cells, in the hypodermal layer. The average number of these sclereids per mg. of black pepper is 4,585 ± 450.

4. The number of sclereids per milligramme of the " husk " has been determined by the lycopodium method ; it is found to average 13,230 with a variation of ± 20. Using this value, the amount of " husk " added to ground white pepper can be determined with remarkable accuracy.

5. Finally the proportion of " husk " normally present in black pepper was determined both microscopically by the lycopodium method and also by removing the " husk " from a weighed known number of berries and weighing the " husk " removed. The average amount of " husk " in black pepper berries was found to be 34·7 p.c.

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825.21 III.—THE USE OF A CAMERA LUCIDA FOR MICROMETRY AND COUNTS OF NERVE FIBRES AND OTHER SMALL OBJECTS.

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TWO TEXT-FIGURES.

IT is difficult by the usual methods of micrometry to obtain more than a rough approximation of the axis cylinder or myelin sheath diameters of very small nerve fibres. It has been found that such measurements may conveniently be made by projecting a scale such as that illustrated (fig. 1a) on to the microscopical field by means of an Abbé camera lucida.
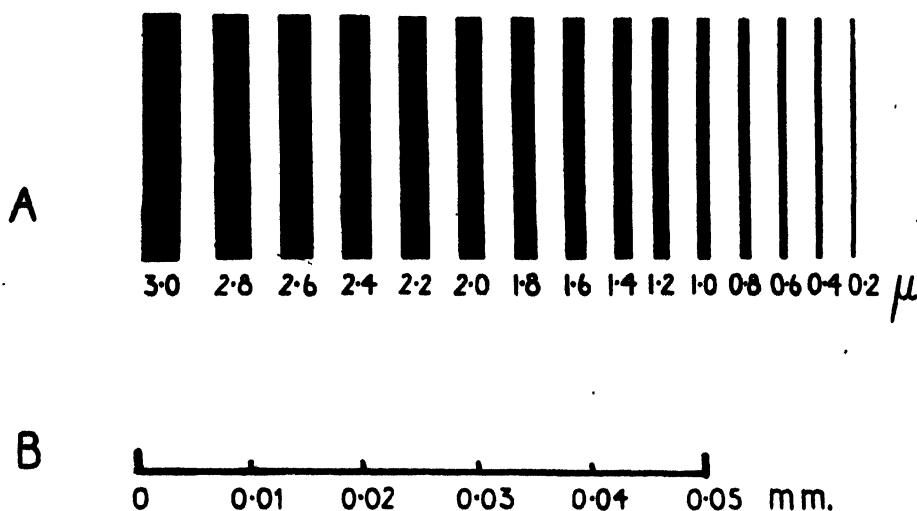
Fig. 1.

A large scale, with lines the thicknesses of which are graded according to the purpose of the investigation, is prepared and then reduced photographically. The precise breadth of the end of each line in the reduced scale is measured under low magnification using incident illumination and micrometers. The reduced scale is now placed under the mirror of the camera lucida; it is calibrated by marking on it the position of the stage micrometer divisions when seen with an oil-immersion objective (fig. 1b), and calculating the diameter in microns of an object with which the thickness of each line would correspond.

In use, the scale is projected on to the microscopical field and moved until the image of the end of one of the lines coincides in diameter with the image of the nerve fibre or other object to be measured. It is possible by this method

to estimate, with a degree of accuracy satisfactory for most purposes, the size of objects almost as small as 0.2μ, that is, near to the limit of resolving power of the microscope. Because of optical distortion it is, of course, impossible to give exact measurements of very fine fibres, but these may readily be classified in size groups ; thus, those approaching a diameter of 0.2μ may be separated from those approximating more closely to 0.4μ, and 0.4μ fibres from 0.6μ fibres.

The square shown in fig. 2 may be used with the camera lucida at the same time in order to divide the microscopical field into sections, each of which is
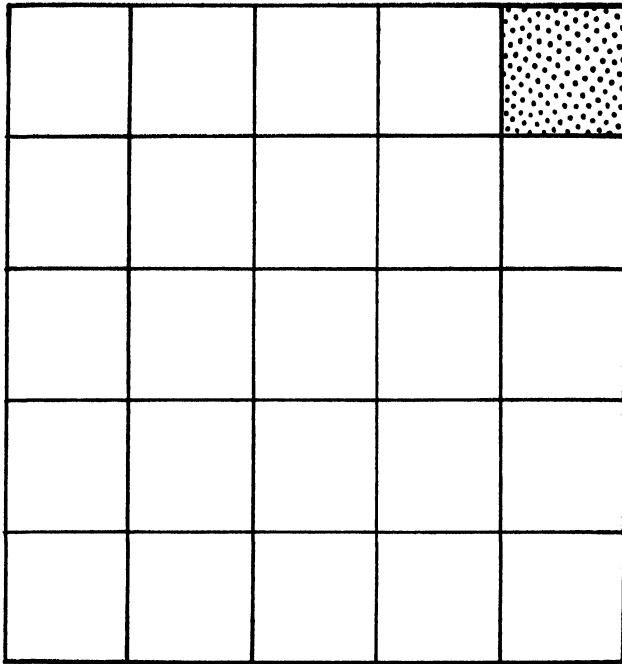
Fig. 2.

examined in turn. In numerical estimations, the number of fibres appearing in each small square may be counted directly, if their number is sufficiently small, and the total number calculated. If the fibre density is so great that difficulty is experienced in making direct counts, the position of each fibre is lightly marked in pencil (as shown in the upper right-hand square) and the marks are counted later. An extensive region may thus be covered, taking fields the size of the large squares one by one ; or representative fields may be chosen, an estimate of the average fibre density obtained, the area of the tract or region determined by drawing its outline with a camera lucida and low-power objective, and the total number of fibres calculated from these data.

These simple methods have proved of value in estimating the number and measuring the diameter of fibres in certain tracts of the central nervous system. They should be equally satisfactory if applied to measurements and counts of small objects other than nerve fibres.

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IV.—THE DETERMINATION OF CALCIUM IN HISTOLOGICAL SECTIONS.

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Two Plates.

THE methods commonly used for the determination of calcium deposits in histological sections fall into two groups. Those of the first group are based on the conversion of the calcium salt into the salt of another metal. This newly formed compound can then under certain conditions give a darkly coloured precipitate by itself, or can be further converted into still another, black or brown compound. Thus von Kossa (1901) described a method in which the calcium salt was treated with a silver nitrate solution and the resulting silver salt reduced by bright light. Stoelzner (1905) and Macallum (1912) advocated methods, based on the change of the calcium salt into the salt of lead or cobalt and the further treatment of this compound with ammonium sulphide, resulting in the formation of a black cobalt- or lead-sulphide.

The second group of methods makes use of the ability of certain dyes to form lakes with metals. Some of these lakes are insoluble in water, alcohol, and other reagents used in the histological treatment of sections, and exhibit a colour characteristic of the metal forming the lake. A number of these dyes have been found to give such characteristic and insoluble calcium lakes. Grandis and Mainini (1900) found that purpurin in a saturated alcoholic solution gave a rose-red lake with calcium; Salomon (1914) used anthrapurpurin, which gave a purple reaction and Schaetz (1926) obtained a brownish precipitate with Gallein. Madder has long been used in the demonstration of bone growth by feeding animals with the powdered root, the staining principles in it being alizarin, purpurin and purpurin-3-carboxylic acid (Richter, 1937).

Schuscik (1920) and Cameron (1930) critically investigated these methods and found that von Kossa's and Stoelzner's methods were specific neither for calcium nor, as was thought by von Kossa, for phosphates. Similar reactions were obtained with phosphates and carbonates of strontium and barium, of ferric iron, and of copper, and with the carbonates, oleates, and oxalates of calcium. It must further be pointed out that the black precipitates obtained with these methods obscure histological structures in the section (fig. 1); they can, moreover, by confusion with other substances, like melanin, lead to wrong conclusions (fig. 4).

Schuscik found that, of the lake-forming dyes, purpurin and anthrapurpurin

were specific for calcium. But these two dyes are insoluble in water and only moderately soluble in alcohol. As they deteriorate fairly quickly in solution, in prolonged investigations they have to be made up frequently. Cameron, therefore, recommended a water-soluble derivative of alizarin, alizarin sulphonate (alizarin red S) ; but this dye gives a powerful general coloration of the section and therefore needs differentiation in both alkalis and acids. Richter (1937), who investigated bone staining by feeding, recommended purpurin-3-carboxylic acid (pseudopurpurin), a water-soluble derivative of purpurin. This dye, however, is not commercially available and has to be prepared in the laboratory. Gallein was found by Cameron not to be specific for calcium and the solutions of it soon deteriorate.

All the methods outlined have limitations as to their sensitivity towards calcium. They reveal calcium deposits ; but do not show masked calcium or very small amounts of calcium (as, for instance, the calcium in the cell nuclei). A truly microchemical reaction was described by Cretin (1924) ; this method, however, is somewhat drastic and not easily performed.

It was thought that a simpler method of demonstrating calcium deposits, needing no differentiation, would be of value, even if it were less sensitive than Cretin's, provided it was specific.

Twenty-two dyes of the hydroxyanthraquinone, hydroxyketone, xanthene, and oxazine groups of dyes * were investigated. These groups were selected for their known ability to form lakes with metals. Considerable difficulties were encountered in obtaining dyes of sufficient purity commercially and there was also a considerable difference in the behaviour of the commercially obtained dyes from different suppliers.†

The dyes were tested on sections through the heads of newly born rats and on sections through young trout. The objects were fixed in 95 p.c. alcohol or neutralized formalin, embedded in paraffin wax, and cut into sections of 10μ thickness. The sections were freed from paraffin, brought down to 70 p.c. alcohol, rinsed in distilled water, and stained for 10 minutes in the dye solutions.

The solutions employed were saturated aqueous solutions of the water-soluble dyes ; alcoholic or alkaline solutions of the remainder. Of the dyes tested only one, gallamine blue, showed marked advantages over the others.

Gallamine blue (C.I. No. 894) is an oxazine dye, which was introduced as a nuclear stain by Becher (1921). The free base is only very sparingly soluble in water, but the bisulphide compound and the hydrochloride of the base are freely so. The bisulphide was obtained from Messrs. T. G. Gurr, London, and the hydrochloride from Clayton Aniline Co., Ltd., Manchester. The two compounds behaved similarly towards calcium.

Gallamine blue in a saturated aqueous solution (about 0·1–0·2 gm. of the

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* The dyes investigated were : hydroxyanthraquinones : alizarin, alizarin sulphonate, anthragallol, purpurin, anthrapurpurin, alizarin cyanin 2R, alizarin cyanin 3R, alizarin saphirol SE, alizarin saphirol B, anthracene blue SWX, anthracene blue W, alizarin blue S, alizarin cyanin green ; hydroxyketones : naphthazarin, naphtopurpurin ; xanthenes : gallein, coerulein ; oxazines : Meldola's blue, gallocyanin, gallamine blue, coelestine blue, prune E.

† I am greatly indebted to the Dyestuffs Division, Imperial Chemical Industries ; the Clayton Aniline Co., Ltd., Manchester ; and Messrs. Geigy, Ltd., Basle, for preparing samples of the dyes used in this investigation.

dye were boiled in 100 ml. of distilled water for 5 minutes, cooled and filtered) gave a brownish-purple reaction with calcium; the rest of the tissues, particularly striped muscle, was stained blue but keratin deep purple. The differences in colour between the calcium and the other tissues were very pronounced.

It was found, however, that the colour reactions of both calcified and other tissues changed with the age of the solution. The colour of the calcium changed more and more towards a yellowish-brown, the rest of the tissues to green. Moreover, if a section was left in the solution for some time, small deposits of calcium were dissolved because of the acidity of the distilled water; this difficulty had been pointed out by Schaetz.

Buffered solutions were therefore prepared. The buffer solution used was the borax–boric acid buffer of Palitzsch adjusted to a pH 7·6 (Sol. A : M/20 borax solution by dissolving 19·1 gm. $Na_2B_4O_7.10H_2O$ in 1000 ml. distilled water. Sol. B : a solution containing M/5 boric acid and M/20 sodium chloride by dissolving 12·4 gm. of H_3BO_3 and 3 gm. of NaCl in 1000 c.c. distilled water. 15 ml. of solution A were mixed with 85 ml. of solution B to give a buffer of pH 7·6). The staining solution was prepared by boiling 0·2 gm. of the dye in 100 ml. of the buffer. The buffered dye gave a sharp blue reaction with calcium and left the rest of the tissues unstained. The solution remained unchanged for about 2 months, but needed occasional filtering. After 2 months the staining of calcium became somewhat weaker, but the shade remained unchanged. Even after one year the solution was found to be still usable.

The staining obtained with both the aqueous and buffered solutions was affected neither by distilled water during 10 minutes washing nor by alcohol, xylene, or alkaline solutions. It disappeared in acid solutions. It did not fade in Canada balsam. If the staining was not prolonged unduly no differentiation was necessary, but if the sections were left in the buffered solution over-night or longer the other tissues took up a purplish stain. This could, however, be removed with a 0·01 p.c. solution of KOH without affecting the calcium stain.

To test the specificity of the stain, precipitates of calcium, magnesium, manganese, and ferric salts were obtained in films of collodium in a manner indicated by Cameron. These films were then stained with the gallamine blue solution. Of these four metals only calcium gave a water- and alcohol-insoluble colour; ferric salts gave a purple lake, different in colour from calcium and soluble in water; magnesium and manganese gave blue lakes, which, however, were dissolved in alcohol. These metals were chosen because they are the metals most commonly associated in tissues with calcium.

The staining of calcium deposits with gallamine blue appears to be a surface reaction, for the edges are distinctly deeper stained than the centre. Prolonged staining did not result in a noticeably heavier coloration, nor does the depth of the staining depend on the amount of calcium present.

The staining, though showing up calcium well (fig. 2), was sufficiently transparent to allow the demonstration, with suitable contrasting dyes, of other histological elements present in the deposits (fig. 3). Nuclei, for instance, can be stained with an alkaline safranine solution. The aluminium lake of alizarin sulphonate was also found to be a satisfactory counter stain.

Fig. 1.

Fig. 2.

It therefore appears that the methods of calcium determination with lake-forming dyes offer some advantages over those based on the conversion of the calcium salt into the salt of another metal. Gallamine blue in its water-soluble forms can be used in easily prepared solutions. These solutions give sufficiently specific reactions with calcium deposits. It is, however, possible that further investigations may show that other lake-forming dyes may give similar or better results in the determination of calcium.

SUMMARY.

(1) Twenty-two lake-forming dyes were tested for their ability to form specific lakes with calcium. Of these, gallamine blue was found to form water- and alcohol-insoluble lakes.

(2) Aqueous or alkaline buffered solutions can be used. The first gave brownish-purple, the latter blue lakes. With unbuffered solutions, uncalcified tissues gave colours contrasting with calcified tissues ; the buffered solution left uncalcified tissues colourless. No differentiation is normally necessary. The buffered solution has advantages over the unbuffered.

(3) The sensitivity of this reaction seems to equal that of the other known methods, with the exception of Cretin's microchemical method. It is thought to be sufficiently specific for most histological work. Suitable counterstains can be employed.

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DESCRIPTION OF PLATES.

PLATE I.

Fig. 1.—Section through the head of a newly born rat (× 93). Region of vertebræ. The bone has been stained with silver-nitrate solution (v. Kossa's method). Calcium is shown black.

Fig. 2.—Section through the head of a newly born rat (× 93). Region of vertebræ. The bone stained with gallamine blue in buffered alkaline solution. The section counterstained with the aluminium-lake of alizarin red S. Calcium is stained blue ; nuclei, muscle, etc., in various shades of red and orange.

PLATE II.

Fig. 3.—Inset of fig. 3 further magnified (× 420). Ilford filter Micro. 1 has been used in taking the photograph to suppress the blue and intensify the red. The photograph shows osteoblasts (o) visible within the bone.

Fig. 4.—Section through the head of a young trout (× 93). The bone has been stained with silver-nitrate solution (v. Kossa's method). This section contains both melanophores (M) and bone (B). Both are black in the section.

Fig. 5.—Section through the head of a young trout (× 93). The bone has been stained with an alkaline buffered solution of gallamine blue. This section contains both melanophores (M) and bone (B). The bone is stained blue and contrasts sharply from the black pigment cells.

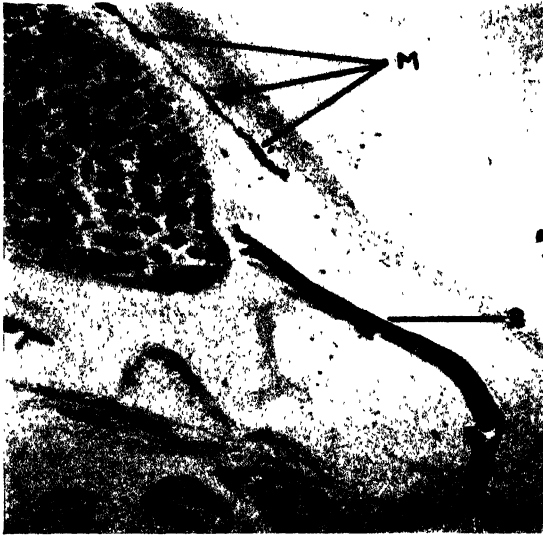The photomicrographs were taken by Mr. P. D. Mumby, F.R.M.S.

Fig. 3.

Fig. 4.

Fig. 5.

V.—A SYNTHETIC RESIN WHICH HAS UNUSUAL PROPERTIES. 535.827.1

By G. D. HANNA, F.R.M.S.

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IN February and March, 1931, I was working with synthetic resins, endeavouring to find one which would have suitable properties for high microscopic resolution and other necessary features. Many forms found mentioned in the literature of such substances were tried without a promising lead, when it was decided to attack the possibilities of sulphur again. A previous attempt to use a combination of aniline, sulphur, and formaldehyde at first indicated a desirable mounting medium, but soon thereafter it was displaced by the napthalene-formaldehyde resin which was named " Hyrax " and which has been used widely.

Upon re-studying the old sulphur experiments and a subsequent limited search of references in chemical literature it was decided that there was a chance of success in a different way than I had tried before. Sulphur is prone to combine with many organic substances, but usually yields very dark compounds, entirely useless from the microscopist's standpoint. However, it was found that sulphur reacts with phenols and related substances and it was believed that they might form a starting-point toward a satisfactory product.

Preliminary experiments occasionally yielded a resin of rather promising possibilities and some very desirable properties. It was made of sulphur, phenol, and a small amount of sodium hydroxide. Its solubility in ethyl alcohol and acetone and high index of refraction made it appear especially suitable for mounting preserved plankton. Pressure of other duties made it necessary to drop the work before a satisfactory procedure had been worked out for making the resin. Some small samples were distributed to fellow workers, under the name " Pleurax." *

Through their persistent requests, the work has been resumed and with the above historical background, the following paragraphs will give a progress report up to March, 1948.

After many trials it was found that the following proportions give a very fine product :

Sulphur, 40 gm. ; phenol, 100 gm. ; sodium sulphide, 2 gm.

Powdered sulphur is used. Phenol is in crystalline form and nearly colourless ; it may have a faint pink tinge without detriment. The sodium sulphide is the anhydrous flake form manufactured by Eastman Kodak Company, for

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* This name was derived from the diatom genus *Pleurosigma*, a species of which was used to make comparative tests of resolution of the resin.

photographic purposes.   The mixture is heated slowly at first in a flask equipped
with a straight vertical tube about 15 inches high to serve as an air condenser.
The joint must be ground in.   If a rubber or cork stopper be used only a dark
product will result.   The heating must be gentle and if no method of stirring
is provided, larger quantities than those indicated may become dark, apparently
due to too high a temperature adjacent to the flask wall.   Large quantities
of hydrogen sulphide are evolved, and some means must be provided to trap
this or the work should be done under an efficient fume hood.

The quantities of chemicals specified provide an excess of phenol inten-
tionally.   This is to ensure the using up of all of the sulphur in the reaction.
As the heating progresses a small sample taken from the flask on the end of a
rod is dissolved in 95 p.c. ethyl alcohol.   The heating must continue until such
a sample yields no precipitate of " milky " sulphur.

When this stage is reached the excess phenol must be removed by one means
or another.   A satisfactory way is to pour the contents of the flask into a
shallow vessel or pan and heat until a small sample taken on the end of a rod
becomes a brittle solid on cooling.   The total time of heating is about 8 hours.

The resin cools to a light yellow, amorphous, brittle resin having a slightly
greenish cast.   It adheres very tenaciously to glass slides and covers.

Solubility in 95 p.c. ethyl alcohol and in acetone is complete and gives a
thick fluid resin suitable for mounting purposes.   Thus, a drop of this placed
on a slide and with a drop of alcohol-preserved plankton stirred therein can be
made into a beautiful lemon-yellow coloured mount in a few moments.   The
excess solvent is removed by gentle heat in the usual way.   So far as I know
it has not been tried in other branches of microscopy.   *iso*Propyl alcohol
appears to be an even better solvent.   The usual solvents for balsam are not
satisfactory.   Water decomposes the resin and precipitates sulphur.

A hollow cell was made of three pieces of plain parallel glass and some of
the resin was melted in this to form a prism, the apex of which had an angle of
48° 27′ 30″.   The index of refraction was then determined on a spectrometer
for two wavelengths.   The results are believed to be accurate in the second
decimal place.   More precise instruments for such measurements were not
readily available.   The result for the sodium (d) line was 1·7497 and for the
mercury green line 1·770.

From these figures it is apparent that this resin has an exceedingly high
index of refraction and the dispersion is not excessive.   I know of no readily
usable material which has so high an index.   " Hyrax," for instance, measured
in the same hollow prism and under the same conditions showed 1·7149 for the
mercury green line.

Canada balsam is usually cited as $(n)_D$ 1·52 and may vary with different
lots.   All resins used for mounting media naturally show the highest index
when all volatile solvents are removed.   This is difficult to achieve in some
cases and especially when the nature of the object makes gentle heating unsafe.

The question of permanency of any mounting medium is one of paramount
importance and one which is obviously very difficult to answer.   Probably
there is no answer except by making test slides' and waiting for a specified
number of years (say 100) and see what happens.   In the case of Pleurax, all

that can be said at this time is that test slides made in 1932 had not changed in any noticeable feature in 1948.

There are two special reasons for choosing a mounting medium of high index of refraction. First and of greatest importance is the optical law which states that the visibility of a transparent object is directly proportional to the difference in index of refraction of it and the material immediately surrounding it. Thus, a piece of glass mounted in a transparent medium of the same index of refraction could not be seen. And if a glass had an index of 1·0 for all wavelengths it could not be seen in air. It is for this reason that " non-reflection " coatings of a low index material, such as magnesium fluoride, is placed on certain optical elements. We " see " glass only because its index is higher than 1·0. Obviously if the surface can be reduced somewhat toward that figure it will become less visible. The converse is likewise true. This is not a full explanation of the phenomena of non-reflection coating, which becomes highly involved in mathematics in the final analysis, but is cited merely as one illustration of the importance of differences in indexes of refraction. The same law applies to resolution of fine structure.

Much has been said regarding the importance of using light rays of short wavelengths (and, recently, electron beams, which are far shorter), in order to increase resolution. The importance of increasing the difference in index of refraction between the object and its surrounding has not received as much attention as it deserves. The facts, of course, have been known for a great many years and certain specialists, especially diatomists, have explored the possibilities extensively. It is worthy of note, however, that the incomparable fine rulings made by the Australian, Grayson, were done on films of realgar because that material has an index of refraction of about 2·4.

The second important reason for having the greatest possible difference in index of refraction between the object and its surroundings is connected with an old familiar optical law concerning vertical thicknesses. Nearly everyone knows that an object on the bottom of a pool of water seems to be closer than it actually is. This is due to the fact that the apparent thickness of a layer of material is less than a similar layer of air in direct proportion to the indices of refraction. One method of measuring index of refraction is based upon this law. If a cell of known depth be made on a microscope slide (it can be measured with the graduations on the fine adjustment) and then be filled with the material to be measured and the apparent depth obtained by focusing first on the top of the inner wall of the cell, then on the bottom, the actual depth divided by the apparent depth is the index of refraction. The method is not very accurate, but does give a striking demonstration, even with water (index 1·33). This is of extreme importance in microscopy, but it is rarely mentioned in the literature. An inviolable law in optics is that the axial magnification of a lens is equal to the square of the lateral magnification. Thus, if the object be a hemisphere of 1·0 mm. diameter and the lateral magnification be 4·0, the aerial image will be a hemi-ellipsoid 4·0 mm. diameter and 16 mm. high or long. This is easily demonstrated with any microscope and a piece of ground glass.

This greatly attenuated axial magnification is normally taken care of in

visual observation by constant manipulation of the fine adjustment. In photo-micrography, however, the emulsion layer cuts across this aerial image and the "depth of focus" is often very disappointing, especially in high-power work. It is here that the value of a high index mounting medium becomes greatest.

Pleurax has a strong lemon-yellow colour. Thus it acts, in a way, as its own colour filter, although it is highly transparent to green rays. No quantative tests have been made as to its transmission and absorption. Specimens which are normally differentiated by staining technique might not be improved at all by its use. However, the total solubility of the resin in ordinary ethyl alcohol might be expected to make it feasible to eliminate the usual "clearing" of tissue sections entirely.

Nothing is known of the stability of stained material mounted in it. It has been noted that certain adhesives used to attach objects to slides or covers, such as gum tragacanth, are dissolved or decomposed by the resin. This may be a decided disadvantage in its use for certain applications.

ABSTRACTS

MICROSCOPES AND MICROSCOPY.

Phase-contrast Microscopy.—L. BERTI (" Teoria della microscopia in contrasto di fase—Theory of Phase-contrast Microscopy," *Il Micro Cimento*, 1948, **5**, No. 3). A theory of the phase-contrast microscope is developed, taking into account the position of the light source, the presence and magnitude of aperture and field stops, the structure of the specimen, and the aberrations of the optical system. A full-contrast image of a phase specimen can be obtained by pure interference due to the superposition of the light wave modulated by the specimen and a coherent reference wave. The mathematical theory of such an ideal interference microscope proves that the Zernike method is a useful approximation to the full interferometric one. Simple and general mathematical relations show how the different parameters involved are responsible for the observed phase-contrast phenomena. **B. O. P.**

Polarized Light Microscopy.—W. V. COLE (" Polarized Light in Photo-Microscopy," *J. Biol. Phot. Ass.*, 1948, **16**, No. 4, 147). A technique is described in which polaroid lenses are used in conjunction with photomicrographic equipment to obtain photographs of histological specimens. These included jejenum (rabbit), tonsil (human), thyroid (rat), and lymph node (human). The freezing and paraffin method of sectioning were employed. Sections were cut at 10μ and stained by the spot plate method and routine paraffin staining. Thionin, hæmatoxylin, and gallo-cyanin were the nuclear stains employed and eosin was the only counter-stain used. It was found that the use of polaroid lenses did not appreciably aid in visual observation in routine microscopy, but for photographic purposes cellular detail and contrast were improved by using a yellow filter and a polaroid lens in front of the light source. The most efficient optical system consisted of a 3 mm. 0·95 N.A. apochromatic objective in conjunction with a $\times$ 10 compensating eyepiece. **B. O. P.**

New Microscope Objective.—P. RAMSTHALER (" Ein neues Mikroscops—objectiv mit grossen Arbeitsabstand fur Auflichtuntersuchungen—A New Microscope Objective with Long Working Distance for Incident Illumination," *Mikroscopie*, 1946, **1**, pts. 1–2, 26). An apochromatic microscope objective has been designed working at an N.A. of 0·20 and magnification of $\times$ 20, with the exceptionally long working distance of 23·4 mm. The lens system is intended for use in optical measuring instruments in conjunction with Huyghens' eyepieces, but may have applications in other fields. Diagrams are given comparing the new system with the conventional type of objective and showing the spherical aberration curves for the C, F, and D wavelengths, all of which lie within the optical tolerances. **B. O. P.**

Colour Microphotography.—A. GRABNER (" Mikrofotografie in Farben—Colour Microphotography," *Mikroscopie*, 1946, **1**, pts. 1–2, 29). A full account of methods for obtaining colour photomicrographs. Types of microscope and camera are

discussed and methods of focusing the image considered. Exposure can be estimated photo-electrically, by means of test exposures, or by calculation. The importance of the light source and the method of illumination of the object is stressed. Photo-micrographs of sections of the human scalp and lung are reproduced in colour.

<div align="right">B. O. P.</div>

Microscopy.—F. BRAUTIGAM (" Die Entwicklung und der gegenwartige Stand der Mikroscopie—The Development and the Present State of Microscopy," *Mikroscopie*, 1946, 1, pts. 1–2, 56). The growth of microscopy is described under various headings, such as monocular, binocular, and stereomicroscopy, incident light microscopy and the polarization microscope. Contrast in the microscopic image can be enhanced by embedding, staining, dark-field examination, optical staining, fluorescence-microscopy, and phase-contrast, whilst resolution can be increased by the use of the ultra-violet and electron-microscopes. Each of the above techniques is described and the increasing use of microscopy in many fields of investigation is emphasized.

<div align="right">B. O. P.</div>

Macro-photography.—A. GRABNER (" Schärfentiefe und Abbildungsmassstab bei der Lupenfotografie—Depth of Focus and Image Magnification in Macro-photography," *Mikroscopie*, 1946, 1, pts. 3–4, 106). Expressions are derived for the depth of focus and image magnification obtainable with camera lenses used for macro-photography in terms of relative aperture, focal length of objective, image distance, and the diameter of the largest permissible circle of confusion. From these tables are constructed enabling the maximum allowable magnification corresponding to a given relative aperture and depth of focus to be obtained and the results are expressed graphically. Field dimensions are also worked out for the various magnifications corresponding to standard sizes of photographic plates or films.　　　B. O. P.

Condensers.—CITTERT, P. H. (" Einige Bemerkungen uber den Einfluss des Kondensors—A Few Observations on the Influence of Condensers," *Mikroscopie*, 1948, 3, 92–5). Two methods of illuminating an object are considered: (*a*) directly by means of an extensive light source; (*b*) by means of an image of this light source formed in the object plane by a lens. It makes little difference which method is used. The degree of coherence between the resulting light-waves in the different points of the plane depends only on the angular aperture of the incident light and is independent of the manner in which it is received. The condenser thus has very little influence on the quality of the image and an exactly similar picture is formed in the image plane if the object is illuminated directly from the source at the same angular aperture. From consideration of an illuminated grating ruling it follows that the Helmholtz and Abbé theories of the microscopic image lead to identical results.　　　B. O. P.

Phase-contrast Microscopy.—ZOLLINGER, H. V. (" Phasenmikroscopische Beobachtungen an Zellkulturen—Phase-microscope Observations on Cell-cultures," *Mikroscopie*, 1948, 3, 1–11). Evidence of the usefulness of the phase-contrast micro-scope in cytological fields is examined and its achievements up to the present day are summarized. A brief explanation is given of the idea of optical phase-difference and its importance in phase-microscopy. In the examination of cell cultures the different elements in living cells are made very clearly recognizable. Changes in progress can be closely observed and photographically recorded over a long period. Moreover, the observations of earlier workers, which were originally obtained on cell suspensions, can be verified and confirmed with cell cultures. Brief mention is made of the insight into the physico-chemical structure of the cell elements which has, so far, been revealed by the use of the method.

<div align="right">B. O. P.</div>

Universal Stage.—KOHLER, A. (" Die Bedeutung des Universal-Drehtisches nach Federov in der Mineralogie und Petrographie—The Importance of the Federov Universal Stage in Mineralogy and Petrography," *Mikroscopie*, 1947, **1**, 174–87). The development and method of use of the Federov universal stage in the identification of the optical properties of minerals are described, taking the Abbite-Anorthite series as an example. Graphical methods are discussed and a modern form of the stage is illustrated. B. O. P.

Flat Field Objectives.—RAMSTHALER, P. (" Über Planachromate—On Flat-Field achromatic Objectives," *Mikroscopie*, 1947, **2**, 55–8). Three types of flat-field achromatic objectives, the × 4 0·10 N.A., the × 10 0·20 N.A., and the × 40 0·65 N.A., are illustrated in section, together with curves showing the enhanced field-flatness resulting from their use. A pair of comparative photographs of a stained blood-smear demonstrates the resulting improvement in the final image. B. O. P.

Micro-hardness Testing.—RAMSTHALER, P. (" Über einen neuen Mikroharte-prufer—On a New Micro-hardness Tester," *Mikroscopie*, 1947, **2**, 345–52). A micro-hardness tester is described which has been developed for use with the Reichert Universal Microscope, Model Me F. The apparatus employs an objective of 0·65 N.A. giving a primary magnification of × 40, which is mounted on a slide together with the Vickers diamond pyramid point used for making the impression. There are two positions for the slide. In one, the diamond point lies on the optic axis of the instrument and the load is applied by the deformation of steel tapes, its value being read from a moving scale imaged in the measuring eyepiece by a special optical system. In the second position of the slide the microscope objective is brought into use to form an image of the impression and to enable its size to be determined by the aid of the eyepiece micrometer. It is intended to develop a micro-hardness tester employing a dry objective of 0·90 N.A., having a primary magnification of × 63, and giving a total magnification of × 800. B. O. P.

Micro-hardness Testing.—ONITOCH, E. M. (" Über die Mikroharte der Metalle —On the Micro-hardness of Metals," *Mikroscopie*, 1947, **2**, 131–94). A brief explanation of the idea of hardness is followed by a survey of known micro-hardness testers and a short description of the Reichert form of the instrument. The nature of micro-hardness and its calculation is described and the importance of the exponent *n* in the Meyer power law is pointed out. A new hardness criterion is suggested, embracing both micro- and macro-hardness measurements. Examples of measurements on various materials are taken from the literature and used to emphasize the importance and practicability of micro-hardness testing methods and to bring out the connection betwee micro- and macro-hardness. B. O. P.

Phase-contrast Microscopy.—OSTERBERG, H. (" The Multipupil in Phase-Microscopy," *Journ. Opt. Soc. of Amer.*, 1948, **38**, 685–8). Auxiliary lenses may be employed in a modified phase microscope to form additional images of the condenser diaphragm. The images of the condenser diaphragm are called multipupils. They can serve to increase the flexibility of the phase-microscope, because a diffraction plate may be placed at any one or all of the multipupils. Multipupil systems serve as effective means for phase-microscopy. The bipupil system is of most practical interest because it combines the required flexibility with simplicity. It is shown on general theoretical grounds that the multipupil system is equivalent to a single system

of phase-microscopy whose overall magnification is equal to the product of the magnifications of the multipupil system and whose pupil function is the product of the complex pupil functions associated with each of the multipupils. The physical interpretation of the composite pupil function as a product is that the optical paths through corresponding areas of the multipupils are additive, while the corresponding amplitude transmissions are multiplied. The method of proof serves to extend the theory of phase-microscopy to multipupil systems. B. O. P.

Ultra-violet Microscopy.—FOSTER, L. V., and THIEL, E. M. (" An Achromatic Ultra-violet Microscope Objective," *Journ. Opt. Soc. of Amer.*, 1948, **38**, 689–92). Microscopes have been used with monochromatic illumination because achromatism in the ultra-violet has not been common. The authors have designed and made a few different objectives achromatized for a band width of about 100Å in the region 2700Å. These objectives make use of fused quartz and fluorite. They can be used in other regions of the ultra-violet spectrum, but require re-adjustment of focus. The system treated in greatest detail has a focal length of 2·5 mm. with a numerical aperture of 0·85 and magnification of × 71. B. O. P.

Bacteria and Phase Microscopy.—O. W. RICHARDS (Phase Microscopy in Bacteriology," *Stain Technol.*, 1948, **23**, 55–64, 15 refs. ; 1 plate and 1 text-fig.). The theory and method of use of the phase-contrast microscope are explained with special reference to bacteriology. The microscope can be used to examine unstained growing cultures in Petri dishes, even with the oil-immersion lens. Flagella are detected for the first time on the living spore of *Ashbya gossypii*. Some of the larger bacteriophages have been seen with 0·1A+0·25λ diffraction plate. Micro-organisms show sharp edges under the phase microscope and measurement of unstained living cells now becomes possible. *Bacillus megatherium* has an average width of 1·0μ and *B. cereus* 1·1μ with very small variations. Observations on locomotion confirm those reported by Pijper. Stained preparations of low contrast are seen with enhanced contrast by phase microscopy. G. M. F.

HISTOLOGICAL AND CYTOLOGICAL TECHNIQUE.

Leishman's Staining.—R. H. BLACK (" Leishman's Stain adapted for Use with Histological Sections," *Ann. trop. Med. Parasitol.*, 1948, **42**, 52–3, 3 figs.). Although Giemsa's stain has often been used for tissue sections, Leishman's stain has as a rule proved unsatisfactory, especially for demonstrating the various stages of malaria parasites. Thin sections are treated for 5 to 10 minutes with a saturated aqueous solution of picric acid; washed in water and stained for 25 minutes in a mixture of 1 part of Leishman's stain and 2 parts of distilled water. This mixture must be freshly prepared. The sections are then washed in running tap-water for 10 to 15 minutes, and mounted in a neutral mounting medium. Malaria parasites in red cells are readily seen and exoerythroryptic forms are stained very deeply. Tissue cells are brilliantly coloured, and in some organs such as the kidney differential staining is marked. The method is not an improvement on Giemsa's stain and there is no differential staining of the chromatin and cytoplasm of the parasites. G. M. F.

Staining Cerebellar Climbing and Mossy Fibres.—P. M. E. CARREA, M. REISSIG, and F. A. METTLER (" Histological Technic for Cerebellar Climbing and Mossy Fibers," *Stain Technol.*, **23**, 65–8, 4 refs.). Rio Hortega's double impregnation method for nerve fibres is employed. Brains are immersed in 10 p.c. neutral formalin and

hardened for at least 15 days. Cross-sections of the cerebellum are cut on a plane, perpendicular to folia, and from these blocks frozen sections are then cut at 15 to 25μ; these sections are collected in a dish filled with distilled water alkalinized by a few drops of ammonia. Sections are then treated as follows: Rinse in distilled water; place in cold 95 p.c. alcohol with 3 drops of ammonia per ml.; warm to 45°–50° C. for 10 to 15 minutes; transfer to distilled water with ammonia, followed by three changes in distilled water. Mordant in 2 p.c. silver nitrate with pyridine (10 to 20 drops per 30 ml. of silver nitrate solution) at 45°–50° C. After about 30 minutes sections assume a light yellow-brown colour; they are then transferred to a weak solution of Rio Hortega's silver carbonate (silver nitrate 10 p.c. 50 vol., sodium carbonate 5 p.c. 150 vol., ammonia water to dissolve precipitate, distilled water 550 vol.) with 5–20 drops of pyridine per 30 ml. of silver carbonate, at 45–50° C. The staining dish should be covered with a watch-glass and agitated gently at intervals of about 2 minutes. When sections begin to show a light caramel-brown colour one section is transferred to neutral formalin for control. The following variants are then tried: (1) Formalin 1 p.c. (2) Distilled water, then transfer to 10 p.c. formalin. (3) Wash in distilled water and tone without reduction.

Sections are placed on a slide and examined under the low power to see if the fibres are stained. If so, a cover-glass is placed over them and examination is carried out with a high dry power ($\times$ 40). If fibres are stained, the whole slide is now placed in water and a stained section saved for final mounting and toning. Untoned sections are better for climbing fibres, toned for mossy fibres.

Toning is carried out in gold chloride solution 1 : 600 till the sections are purplish-blue. If necessary, toning is intensified by warming sections in gold chloride at 45°–50° C. Fix in 5 p.c. sodium thiosulphate; dehydrate in alcohol; clear in carbol-xylol to which a few drops of clear beech wood creosote are added. Mount in balsam.

With this technique cellular structures are lightly stained and all fibres are well demonstrated.   G. M. F.

The Double-embedding of Tissues.—J. O. BROWN (" A Simplified and Convenient Method for the Double-embedding of Tissues," *Stain Technol.*, 1948, **23**, 83–9, 3 refs.). A method of embedding tissues in celloidin and paraffin is described. The specimens are fixed or stained *in toto* and then thoroughly dehydrated. Alcohol 20 p.c. is first used and by 5 p.c. increases (6 to 8 hours each) to 95 p.c. alcohol, at which concentration two changes for 24 hours are necessary. Two changes of absolute alcohol (12 hours each) are followed by alcohol and ether (equal parts); celloidin 2 p.c., 4 p.c., and 6 p.c. are applied for 1, 1, and 2–4 days. The specimen is then placed in an open, paraffin-lined dish containing 6 p.c. celloidin, sufficient to cover the specimen. The paraffin-lined dish with its contents is placed in a large covered dish with enough chloroform to cover the bottom. The celloidin block is removed from the paraffin-lined dish, turned upside-down, and quickly trimmed to remove the excess celloidin. The block is cleared in three changes of generous amounts of benzene (benzol). An amount 4 to 5 times the volume of the block is sufficient. The cleared block is transferred to a saturated mixture of paraffin (47°–50° C. melting-point) in benzene at 37° C. The block remains in this mixture for 24 hours and is then impregnated in three paraffins with melting points of 47°- 50° C., 53°–55° C., and 53°–55° C.   G. M. F.

Impregnation of Nervous Tissues.—J. A. ARCADI (" Impregnation of Oligo-dendroglia in Nervous Tissue kept in Formalin for Many Years," *Stain Technol.*, 1948, **23**, 77–82, 7 refs.). The oligodendroglia in nervous tissue preserved in formalin for

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many years can be impregnated by the following method. The tissue is reconditioned by placing 12–30μ frozen sections in concentrated ammonia (sp. g. 0·90) and by washing them slowly for 24 hours with a 1 mm. stream of water. The fluid is then poured off the sections and the jar refilled with concentrated ammonia. Washing is repeated for another 24 hours. Sections are then again plunged into concentrated ammonia for 7 minutes and incubated for 1 hour at 38° C. in Globus's 5 p.c. hydrobromic acid solution. They are again washed in distilled water and impregnated with ammoniacal silver carbonate solution (5 ml. 10 p.c. silver nitrate added to 15 ml. of 5 p.c. sodium bicarbonate. The precipitate is dissolved in concentrated ammonia and diluted to 50 ml. with distilled water. Impregnation is followed by reduction in 1 p.c. formalin without agitation, fixation in 5 p.c. sodium thiosulphate, dehydration, and mounting in clarite.   G. M. F.

Mordanting.—R. A. POPHAM ("Mordanting Plant Tissues," *Stain Technol.*, 1948, **23**, 49–54, 11 refs.). The use of mordants as a preliminary to staining has received comparatively little attention and the physics and chemistry involved are still highly debatable. Some so-called mordants simply change the pH of the self-mordanting stain solutions or alter the physical state of the cell so as to permit adsorption of the dye. Mordants can (1) precede the stain, (2) be mixed with the stain to form a self-mordanting solution, (3) follow the stain. Often more than one mordant can be used. Different mordants are required for the natural dyes such as brazilin, hæmatoxylin, and carmine, basic synthetic dyes, and acid synthetic dyes. Tissues difficult to stain with Harris's hæmatoxylin may be transferred from distilled water to a mordant of 1/N HCl (82·5 ml. hydrochloric acid in 1000 ml. of distilled water) for 1 hour. After rinsing in distilled water and staining, nuclei and dividing chromatin stain intensely and sharply. Iron, chromium, and zinc salts may be used as mordants for natural dyes. For basic synthetic dyes acid mordants such as 4 p.c. aqueous silicotungstic acid, chromic acid killing fluids, and tannic acid before staining are of value. Weak alcoholic picric acid or iodine solution (1 gm. iodine and 1 gm. potasssium iodide in 100 ml. 80 p.c. ethyl alcohol) mordants basic violet dyes when applied after staining.

For acid synthetic dyes 2–4 p.c. barium chloride, aluminium, or chromium salts followed by slightly alkaline washes of ammonium hydroxide or sodium carbonate are most frequently used. A self-mordanting anilin blue may be prepared by adding small quantities of aluminium ammonium sulphate to the dye. Tissues difficult to stain with alcoholic fast green FCF, especially following iron-hæmatoxylin, often dye well if first mordanted with a mixture of 1 part of saturated citric acid in 95 p.c. ethyl alcohol and 1 part 95 p.c. ethyl alcohol.   G. M. F.

Glycogen in Tissues.—R. E. MANCINI ("Histochemical Studies of Glycogen in Tissues," *Anat. Rec.*, 1948, **101**, 149–56, 20 figs., 19 refs.). Tissues of man and various animals were fixed in Zenker's fluid or in Bouin's fluid with alcohol. Other pieces from the same tissues were frozen in liquid air and dried in a vacuum at −30° C. in Gersh's apparatus. Smears of vaginal epithelium, blood, and exudate were treated with a similar technique. Histochemical study of glycogen was carried out on the smears and on the undenatured deparaffinized sections by using iodine dissolved in a non-polar solvent such as mineral oil. A comparison of the results using chemical fixation and freezing drying permits a determination of the true distribution of glycogen in different tissues, thereby indicating which are the artifacts produced by chemical fixatives.   G. M. F.

Vital Staining of Liver Cells.—W. L. WILLIAMS ("Vital Staining of Damaged Liver Cells. I. Reactions to Acid Azo Dyes following Acute Chemical Injury," *Anat. Res.*, 1948, **101**, 133–48, 4 figs., 39 refs.). When mice are given daily sub-cutaneous injections of 0·2 ml. of 1 in 4 chloroform or carbon tetrachloride for 2 or 3 days and at the same time receive subcutaneous injections of trypan blue or chlorazol fast pink (0·2 ml. of 0·5 p.c. aqueous solution) the dyes are deposited in the damaged hepatic parenchymal cells in the central zones of the liver lobule. In the peripheral zones dye was limited to the macrophages. The dyes uniformly stained the cytoplasm of the damaged cells, but the intracytoplasmic deposition of vital dye within damaged liver cells differed from that seen in macrophages and renal epithelium because of (1) lack of segregation or aggregation into particles or granules, (2) increased sus-ceptibility to solution by water and alcohol, (3) the property of combination with acetone solutions of counterstains to form new colours. G. M. F.

Demonstration of Ketosteroids.—R. J. BOSCOTT, A. M. MANDL, J. F. DANIELLI, and C. W. SHOPPEE ("Cytochemical demonstration of Ketosteroids," *Nature*, 1948, **162, 572**, 3 refs.). Claims have been made that α-hydroxyketones such as deoxycorti-costerone give a purple colour with reduced fuchsin in Feulgen's "plasmal" reaction. In this reaction the material under test is treated with cold saturated aqueous mercuric chloride solution, then with reduced fuchsin. Aqueous mercuric chloride is said to oxidize the hydroxyketone to an aldehyde which reacts with reduced fuchsin. With three pure samples of deoxycorticosterone there was no evidence of oxidation to an aldehyde by aqueous mercuric chloride. This technique cannot therefore be relied upon to demonstrate the cytological location of α-hydroxyketone. G. M. F.

Applying Immersion Oil.—J. S. FAULDS ("Another way of Applying Immersion Oil," *J. clin. Path.*, **1**, 246, 1 fig., 1 ref.). An oil-can (Singer Sewing Machine Company) is ideal for applying immersion oil and saves the microscope, bench, and microscopist's fingers from soiling. G. M. F.

Staining Insect Musculature.—SOL KRAMER ("A Staining Procedure for Insect Musculature," *Science*, 1948, 141). A method is described for revealing the muscula-ture of dipterous and other larvæ and of determining their exact origins and insertions instead of by the usual method of dissection. The procedure is as follows: (1) Bouin's fluid (30° C.), 8–10 hours; (2) 50 p.c. ethyl alcohol, 10 minutes; (3) 70 p.c. ethyl alcohol, 1 hour; (4) 95 p.c. ethyl alcohol, 10 minutes; (5) 0·5 p.c. eosin alcohol, 6–8 hours; (6) return to 95 p.c. alcohol and add oil of wintergreen dropwise to larvæ in 95 p.c. alcohol at hourly intervals over a period of 4–5 days; and (7) transfer larvæ to oil of wintergreen. Stages 6 and 7 should be operated with the greatest care. The Bouin's fluid should be prepared with the formalin neutralized with magnesium sulphate. Larvæ die slowly in this solution and take from 1 to 4 hours. After stage (2) dipterous larvæ are transparent and the fat body, the ventral nerve branches, the alimentary canal, the cephalopharyngeal mouth-hooks, and the tracheal trunks and their branches are distinctly visible. Observations are carried out with a binocular microscope and a spot of light. F. C. G.

Improved Moist Chamber.—KENNETH M. RICHTER ("An Improved Moist Chamber for use in Micromanipulation," *Science*, 1948, **108**, 192). A moist chamber is described made from plastic material wherein the central chamber is kept moist by the exposed edges of rolls of moist filter-paper running along each side of the central chamber; these are held in grooves a little more than half circles and are supplied with water along other drilled channels at right angles to these from the

two ends of the apparatus. These latter holes are also filled with filter-paper and plugged at the outer end. Water is supplied by means of small, drilled funnels situated above these channels. The apparatus can also be used for the study of the effects of diverse water-soluble gases upon cells in the hanging drop.

Adequate diagrams are included.   F. C. G.

Chamber for Micro-manipulation.—KENNETH M. RICHTER (" A Constant-temperature Micro-manipulation Chamber," *Science*, 1948, **108**, 119). The instrument was designed for use with the Chamber's micromanipulator and is constructed entire of sheet Plexiglass; it consists of two units, a moist chamber and a gas-mercury thermostat. An inner chamber surrounding the moist chamber is filled with tap-water and the heating device consists simply of the passage of the electric current through the tap-water filling the inner chamber by means of the manipulation unit electrodes. The heat generated is due to the electrical resistance of the tap-water and the circulation of the water within the inner chamber depends upon the formation and guidance of the convection currents established. A step plate ensures that the roof of the inner chamber is not of uniform height and allows for the rapid diffusion of the warmed water away from the electrodes to the far side of the moist chamber. Controlled temperatures from 18° to 48° C. with a variation of $\pm 0.3°$ C. can be obtained. The thermostat is operated by the expansion and contraction of air in the sealed activating chamber which runs alongside the inner water chamber against a column of mercury in which an adjustable platinum wire electrode drops; the dimensions are such that the mercury column moves approximately 1 mm. for each degree C. change in temperature. Adequate diagrams are included.   F. C. G.

Differentiation of Mesenchymal Derivatives.—M. E. LONG (" Differentiation of Myofibrillæ, Reticular and Collagenous Fibrils in Vertebrates," *Stain Technol.*, 1948, **23**, 69–75, 7 refs.). By this technique mesenchymal derivatives such as myofibrillæ, reticular and collagenous fibres can be differentiated. Tissues are fixed in formol-Zenker for 5–12 hours, followed by the using methods of paraffin embedding. Zirkle's butyl alcohol series, however, is used for dehydration and infiltration with paraffin. The embedding paraffin recommended is " parawax " with 8–10 p.c. of bay-berry wax. The tissue-exposed surface of the paraffin block is soaked in water over-night before cutting serial sections at 3–5μ. Sections are mounted using the dilute albumen method, and the slides, thoroughly dried at 37° C. overnight, are left at 60° C. for 10 minutes to melt the paraffin of the sections. Before staining, the sections are treated with 0.25 p.c. aqueous potassium permanganate, washed in running water and then flooded with 5 p.c. aqueous oxalic acid solution. For reticular staining a 10 p.c. silver nitrate bath is succeeded by an ammoniacal silver carbonate solution, followed by reduction in 1 p.c. neutral formalin, toning in gold chloride (1 : 500 aqueous solution) for 15–30 minutes and fixation in 5 p.c. aqueous sodium thiosulphate. Myofibrillæ, the sacroplasmic lining membrane, and other sarcous elements are stained by Heidenhain's azocarmine solution, adult tissues at room temperature and fœtal tissues at 50° C. Differentiation in phosphotungstic acid (5 p.c. aqueous solution) usually takes from 3 to 6 hours. It should be continued till the collagen fibres are decolorized and the solution should be changed when it reddens. For staining adult tissues light green SF in 1 p.c. glacial acetic acid is used and for fœtal tissues 1 p.c. fast green FCF in the same medium. Three minutes is usually long enough. Tissues are differentiated in 1 p.c. aqueous glacial acetic acid rinsed in distilled water and 95 p.c. alcohol, dehydrated, and mounted in damar or clarite. A discussion of the preparation of ammoniacal silver solutions is included.

Cytochemical Studies of Mitochondria.—G. H. HOGEBOOM, W. C. SCHNEIDER, and G. E. PALLADE (" Cytochemical Studies of Mammalian Tissues. I. Isolation of Intact Mitochondria from Rat Liver; Some Biochemical Properties of Mitochondria and Submicroscopic Particulate Material," *J. biol. Chem.*, **172**, 618–35, 2 pl., 22 refs.). By differential centrifugation of a homogenate of liver cells it is possible to separate various fractions, nuclei, mitochondria, and submicroscopic particulate matter. This separation is carried out by the use of 0·88 M sucrose. Rat livers were removed and forced through a masher fitted with a 1 mm. mesh screen that retained the connective tissue framework. The liver pulp is placed on ice and all subsequent manipulations are carried out at 0–5° C. ; 5 gm. of liver pulp is homogenized in an all-glass apparatus in 0·88 M sucrose (Merck, reagent grade), the total volume of the homogenate being 50 ml. Nuclei, unbroken liver cells, and red blood-cells are separated by centrifuging 40 ml. of the homogenate three times for 10 minutes at 600 $\times$ gravity. In the first of the centrifugations 10 ml. of the homogenate was layered over 1 ml. of sucrose, forming a two-phase system. The combined sediments contained red blood-cells, free nuclei and residual intact liver cells. The supernatant was then centrifuged for 20 minutes at 24,000 $\times$ gravity. The opaque sediment, largely mitochondria, is suspended in 0·88 M sucrose. The isolated mitochondria stain with Janus green B in very low dilutions. Mitochondria contain all the succinoxidase present in the cytoplasm of the liver cell: 34 p.c. of the total nitrogen and 19 p.c. of the pentose nucleic acid of whole liver are in the mitochondria. Submicroscopic particulate matter of approximately 100mμ or greater contains no succinoxidase activity, but the pentose nucleic acid content is high.   G. M. F.

Chromosomes of Gall Midges.—M. J. D. WHITE (" Chromosome Studies on Gall Midges," *Ann. Rpt. Dept. Genet., Carnegie Inst. Wash. Yr. Bk.*, 1946–1947, **46**, 165–9). In the gall midges (Cecidomyidæ) the number of chromosomes in the primordial germ cells and oögonia is very much greater than the number present in the somatic nuclei. In *Miastor metraloas* there are 48 chromosomes in the germ line of both sexes but only 12 in the somatic cells of females and 6 in the males. The male soma is haploid and the female diploid. In the past the degree of ploidy of the various tissues was in doubt. Studies on another Cecidomyid, *Monantropalpus buxi*, suggests that the hypothesis of germ-line polyploidy is untenable and most unlikely for the species. The author states that it now seems more probable that the germ line contains a number of extra chromosomes not represented at all in the soma, rather than that the somatic chromosomes are present in the polyploid condition in the germ line.

In *Miastor*, *Oligarces*, and *Mycophila* pedogenetic reproduction is the main method of reproduction, sexual generations occurring only at long intervals in nature. Under favourable conditions these fungus feeders in the larval condition become sexually mature without undergoing pupation and giving rise by parthenogenesis to broods of larvæ, which live as internal parasites of their own mother and emerge from the dead body to feed on the fungal mycelium before entering the reproductive phase. Under these conditions only females are produced and no adult midges ever appear. During unfavourable conditions, " sexual " larvæ are produced which pupate and develop into adult midges before becoming sexually mature; both males and females are produced and mating must take place before oviposition.

Pedogenetic reproduction is true parthenogenesis, the larval ovaries mature precociously and development of the eggs takes place within the maternal body.

A single maturation division, like any ordinary mitosis, takes place, and in *Miastor* 36 chromosomes are eliminated from the somatic nuclei and produce females. " Sexual " larvæ arise by pedagenesis similarly, but of two kinds, males and females, the former possessing but 6 chromosomes in their somatic nuclei. Apparently sex depends upon the number of chromosomes eliminated from the soma during the cleavage divisions.

The method and times of the elimination of the " E " chromosomes (extra chromosomes) in these species is described and discussed.

In *Monarthropalpus* the elimination process takes place during the first meiotic division when there are two kinds of chromosomes and two kinds of poles to the spindle, one side contains diffuse chromosomes and the passive side of the spindle and the other condensed chromosomes and an active side to the spindle. The result of this division is two kinds of nuclei, one with diffuse chromosomes and one with condensed ones. The cells with the diffuse chromosomes remain in the testes as " residual cells " without forming sperms and the others pass through a second division, which is a simple mitosis and the resulting spermatids develop into mature sperms.

The significance of the " E " chromosomes is obscure; in any particular species they appear to be constant in number, so there is no reason to believe them to be genetically inert to the same extent as the " limited " chromosomes of *Sciara* or the " B " chromosomes of Maize. The author suggests that the genes of the " E " chromosomes do not cease to function when elimination occurs, but that their genic products may persist in the somatic tissue of the embryo or larvæ, exerting a delayed effect similar to the " maternal " effects now known in the genetics of many species or organism. F. C. G.

Polyploidy in Orchids.—O. HAGERUP (" The Spontaneous Formation of Haploid, Polyploid, and Aneuploid Embryos in some Orchids," *Kgl. Danske Videnskabernes Selskab Biol. Meddelr.*, 1947, No. 9, 1–22, 71 text-figs.). The chromosome number of a species can undergo changes during fertilization and may differ remarkably in different ovula found in the same organism. In *Orchis maculatus, L.* embryos develop either *n*, 2*n*, 3*n*, 4*n*, or 6*n*, and perhaps other numbers. Of the spontaneous changes of chromosomes when in nature little is known. These various embryos receive differing numbers of genes from the father and become genetically different.

In *Listera ovata*, *n* may be 16, 17, 18 or 19 and the fertilization process is remarkably easy to follow. The male nuclei are very large and deeply staining and the polar nucleus is dead and shrivelled before fertilization takes place ; as it is never fertilized no endosperm develops and a male nucleus is left over and is to be found lying next to the young embryo. On entry of the pollen tube one synergid is killed and frequently all the nuclei of the pollen-tube enter the embryo-sac. Commonly an embryo-sac receives more than one pollen tube and more than two or three male nuclei will then lie next to the female nucleus, in one case a female nucleus was fertilized by two male nuclei simultaneously. If an ovule does not receive a pollen tube it develops a parthenogenetic embryo which will be haploid.

In *Cephalanthera longifolia L.*, the polar nucleus is early fertilized and develops an endosperm with quite a few nuclei that die quickly, so that the ripe seeds do not contain an endosperm. Haploid parthenogenetic embryos develop also in this species. It is possible that *Cephalanthera Damsonium*, Mill. contain aneuploid forms and the polar nucleus is also fertilized and develops a paucinuclear endosperm which is soon absorbed again. More than one pollen tube enters the ovules in this species and it is a fairly common occurrence that one of the synergids forms an embryo so that the seed contains two embryos. In this species the female nucleus

may develop without fertilization by facultative parthenogenesis if the pollen tube should arrive too late. Other species studied were *Platanthera chlorantha*, Custer, and *Orchis strictifolius*, Opiz (=*O. icarnatus, L.*). F. C. G.

Chromosome Structure.—BERWIND P. KAUFMAN ("Chromosome Structure in Relation to the Chromosome Cycle," *Bot. Rev.*, 1948, **14**, 57–126). In this review the author has considered most aspects of chromosome structure as they appear to-day; it is not complete but designed rather to indicate the present level of our knowledge on the subject. Morphological as well as chemical, histochemical, and physico-chemical methods of investigation are considered; though organization in its various aspects is dealt with adequately, the writer avoids considering the chromosome in relation to the cell itself, a relation which modern research has proved indispensable to a well-considered study of the subject. The chromosome can no longer be considered apart from the other cellular organelles.

The subject is dealt with under the following headings: Organization of the Chromosome; Submicroscopic Organization; Chromonemata; the latter is subdivided into two headings, viz. Numerical Relations and Mechanism and Patterns of Coiling.

The chromosomal sheath and matrix are treated under different headings in spite of a fair body of opinion that the former is but a modification of the latter, if, indeed, at such a level it can be said to exist as a separate structure at all.

The evidence relating to the nature of the matrix, the fast-green-staining component, is fully dealt with, and it is pointed out that although the enzyme ribonuclease will remove this part of the chromosome, conclusions based upon this fact are limited by questions concerning the purity of the enzyme and its specificity of action (pp. 63–64).

Difficulties inherent in the acceptance by a number of cytologists of the theory that the matrix represents the Feulgen-positive coating of the genonema are referable to a series of factors, such as the gradual accretion of thymonucleoproteins by the genomata in prophase, the uniformly coloured metaphase and anaphase chromosomes when not revealed by special treatments, and the assumption that the matrix contributes in gene multiplication and metabolism and must contain thymonucleic acid. The term "chromonema" may continue to serve as a morphological description of the genonema and its associated desoxyribose nucleoproteins.

Chromosomes with diffuse centromeres such as those of *Steatococcus* and *Tamalia* after fragmentation by X-rays do not behave as acentric bodies, but continue to divide, and this consideration coupled with other evidence indicate that it may be a compound body. That the chromonemata traverses this body is suggested by the free separation of these structures in diplo- and polychromosomes. The possibility of a shift in position of a functioning centromere within a chromosome is suggested by Corothers, but her theory that the centromere is merely a temporarily modified chromomere is not supported by the evidence presented, nor by the discovery in certain viviparid molluscs of an intimate centromere-centrosome relationship.

The nucleolar-organizing region occupies a specific position in one chromsome and the nucleolus is seen to displace parts of the chromosome. Disturbances in the normal genotypic control may be assumed to upset the functioning capacity of the organizer, for in certain species the timing of appearance and dissolution of the nucleolus is affected. Nucleoli are, however, sometimes produced at the ends of normal chromosomes of the set, and would indicate the absence of a definitive organizer.

Persisting nucleoli are more frequent in several species of *Salix* at lower temperatures; this leads to the suggestion that the nucleolus is a coacervate.

Heterochromotin appears to effect an influence upon the nucleoli. Other secondary constrictions, it is suggested, represent intercalary heterochromatic regions and this

is borne out by low-temperature experiments on plants and starvation experiments upon animals. Special methods of staining and fixation also reveal these regions, and it is suggested that they may be segments of differential coiling. It is pointed out, however, that our knowledge of these regions is far from complete.

The controversial question of the linear differentiation of the chromonematic thread into chromomere-like segments is fully dealt with. Some authors consider each chromomere to be alloycyclic in relation to others and to show a distinct reactivity of its own to nucleic acid synthesis. Other evidence shows them to be differentially condensed regions of the thread. The hypothesis that the Feulgen-positive bands of the giant polytene chromosomes of dipterous larvæ represent ultimate chromomeres has been contested by two authors at least, but observational evidence lends little support to this.

It is suggested that the essential difference between euchromatin and heterochromatin may depend upon the proximity of chromeres with the same nucleic acid cycle; however, in certain plants different cytological appearances are present in the same nucleus or in different cells of a similar stage. Certain position effects in *Drosophila* depend upon the proximity of a specific portion of heterochromatin.

The various considerations relating to the application of the Feulgen technique in chemical and histochemical analyses are considered in detail, as are likewise methods involving the use of enzymes and other tests.

The disposition and differentiation of nucleic acids and proteins is dealt with under this heading. Much experimental work is listed and should serve as a guide for further research.

The property of birefringence in polarized light of the thymo-nucleic acid molecule and polypeptide chains has not provided any fresh information beyond that already available from studies with the ordinary microscope; the possibility, however, of fixation artifacts resulting in an artificial orientation of the nucleic acid molecules must not be overlooked.

The subject of the number and origin of the chromonemata is still largely an unsettled question and possible differences among species, individuals and types of cells are also complicating factors which prevent formulation of a uniform and general interpretation.

A series of hypotheses concerning the coiling mechanism have been proposed, but there still appears to be no unanimity as to the precise number of chromonemata present in the chromosome at any one particular stage, and conflicting evidence appears from different species and similar material prepared by different techniques. The study of the coiling or spiralization of the individual chromonematic threads presents a problem of the first order.

The paper ends with a bibliography of 443 references.   F. C. G.

REVIEWS

Biology of Pathogenic Fungi, edited by WALTER J. NICKERSON; foreword by
J. G. HOPKINS; in co-operation with R. W. BENHAM, A. L. CARRIÓN, R. CIFERRI,
C. W. EMMONS, J. LODDER, D. S. MARTIN, A. DE MINJER, R. L. PECK, P. RE-
DAELLI, M. SILVA, J. W. WILLIAMS, and F. T. WOLF.—Chronica Botanica
Company, Waltham, Mass., U.S.A. (W. Dawson & Sons, London, W.C.2). 1947.
xx+236. Price 5 *dollars.*

Although it is more than a century since fungi were first shown to be true pathogens,
our knowledge of pathogenic mycology is far less extensive than that of protozoology,
bacteriology, or even of virus diseases. This ignorance has been very largely due to
the lack of exact methods of identification, to difficulties in isolation and propagation,
and to ignorance of whether fungi found in particular lesions are really pathogens or
merely chance saprophytes. In addition immunization and chemotherapy have made
but little headway in the control of fungus infections, while the troubles over ter-
minology have obscured the fact that significant information as to the biology of
certain pathogenic fungi is already available. In an attempt to banish some of
the general ignorance on pathogenic fungi Dr. Nickerson has collected summaries by
a number of pioneers in different phases of this subject, special stress being laid on a
comparison of pathogenic fungi and other micro-organisms.

The book begins with an introduction by W. J. Nickerson in which the restriction
of the pathogenic action of dermatophytes to the skin is critically considered. In
view of the very simple growth requirements for most dermatophytes it is curious
that so many organisms, as for instance *Tricophyton*, should be limited to the skin
and its appendages. It is possible that the zinc content of the epidermis is in part
responsible for resistance to infection by dermatophytes: on the whole pathogenicity
decreases with decrease in nutritional requirements and within a genus which contains
pathogenic and saprophytic species, the pathogens are invariably found to have
the more exacting nutritional requirements. There then follow chapters on the
biology of the pathogenic *Torilopsidoideæ* by J. Lodder and A. de Minjer, on chrom-
blastomycosis by A. L. Carrión and M. Silva, *Ptyrosporum ovale* by R. W. Benham,
and *Coccidioides* by C. W. Emmons. There follows an interesting and useful chapter
on the taxonomic work carried out on fungi in Italy during the years 1941–1945.
The taxonomy of the *Actinomycetales*, for instance, was thoroughly investigated by
Baldacci.

The remaining chapters deal with the chemotherapy of fungus infections by
sulphonamides and antibiotics by F. T. Wolf; a valuable review, with maps, of the
geographical distribution of pathogenic fungus infections by D. S. Martin; the nutritive
requirements and metabolic needs of pathogenic fungi by W. J. Nickerson and J. W.
Williams; Nickerson also contributes articles on the metabolic products, such as
pigments, polysaccharides, and enzymes, resulting from the metabolism of pathogenic
fungi and on the respiration and fermentation of yeast-like fungi and dermatophytes;
R. L. Peck discusses the lipids obtainable from these organisms. Each chapter is

provided with an adequate bibliography up to 1946; and finally the book, which is attractively produced, is provided with author, organism, and subject indexes. This work should do much to stimulate an interest in the pathogenic fungi, for it reveals, very fully, the numerous lacunæ in our knowledge. It is to be hoped that further volumes dealing with pathogenic fungi will be published under the same editorship. Histoplasmosis, for instance, is a subject of growing importance while such general subjects as natural variations and induced mutations in fungi urgently require full and authoritative treatment. Like Oliver Twist, one can only ask for more and hope that the request will be granted.   G. M. F.

The Feeding Organs of Arachnida, including Mites and Ticks.—By R. E. SNODGRASS. Smithsonian Miscellaneous Collections, Vol. 110, No. 10, Publication 3944. Washington, August 18th, 1948. 93 pp., 29 text-figs, 77 refs.

The subject is approached from an evolutionary point of view, the mouth parts of the *Arachnida* being compared with those of the *Kiphosurida*, the latter being treated throughout as a separate group, although it is usually considered as a class of the subphyllum *Arachnida*. The writer states that one of the objects of the present paper is to eliminate entomological terms which have no proper application to arachnid anatomy.

A general discussion of arachnid structure then follows, the embryology being dealt with in detail, special attention being given to the development and relationship of the various parts of the feeding mechanism. In speaking of the labrum, it is pointed out that there is no apparent reason for calling the preoral lobe of an arachnid anything else than a labrum. "Epipharynx," "camerostome," "rostrum," "lingula," "tonguelike" and "styletlike process," all being superfluous terms. The median plate below the cheliceræ and proximal to the labrum is shown to correspond to the epistome of mandibulate arthropods, and terms such as "clypeus," "intermaxillary jugun," and "subcheliceral plate" are therefore discarded. Furthermore, it is shown that the pedipalps are the homologues of the mandibles of the mandibulate arthropods in spite of which some workers refer to them as the "maxillæ," or at least this term is given to the coxæ, which are usually closely associated with the mouth. Though it is morphologically correct to designate the pedipalp coxæ "mandibles," the term is not appropriate in a functional sense, in as much as the pedipalp coxæ do not form true jaws in any arachnid. The writer points out that previous interpretations of parts of the chelate pedipalp are not in agreement with its musculature, thus the basal segment of the chela is not a proximal segment of the tarsus, as the single muscle of the finger is obviously the tarsal muscle of a leg, and tarsal subsegments are never interconnected by muscles.

The term "buccal cavity" is rejected as being anatomically unsound, as a buccal cavity should be within the mouth and not outside it. Pavlovsky and Zarin are incorrect in terming it the "preoral cavity."

Evidence is advanced in support of the theory that the arachnid feeding apparatus has been evolved quite independently and has no relation to the feeding organs of modern *Xiphosurida*. It is stressed that the *Xiphosurida* show mistakable affinities to the trilobites. The author considers it evident that the *Arachnida* and the *Xiphosurida* represent separate lines of evolution within the *Chelicerata*, and that the arachnids have not been derived from a *Limulus*-like progenitor.

In considering the *Palpigradi* or *Microthelyphonida*, the author concludes that the pycnogoni proboscis is a structure independently developed, and unrelated to the mouth cone of the *Palpigradi*, and that if the mouth structure of the *Palpigradi*

really is primitive for the arachnids, then there can be no direct relationship of the *Arachnida* to the *Xiphosurida*, and the coxal lobes of the higher arachnids must have been developed quite independently of those of the *Xiphosurida*. Following discussion of the *Palpigradi*, sections are then devoted to the *Solpugida, Pedipalpida, Ricinulei, Chelonethida (Pseudoscorpionida), Scorpionida, Phalangida* (or *Opiliones*), *Araneida,* and the *Acarina.*

Commenting on the popular idea that solpugid mouth-parts constitute a " beak," the author says he sees no reason for this view, and proceeds to give anatomical substantiation of his contention.

In the course of the section on Ticks, attention is called to the so-called " stylet or tonguelike process " extending forward from the lobe which projects from the sub-cheliceral plate. The writer is convinced that this process is none other than the labrum, corresponding exactly with the labrum of other arachnids : its form suggests the labrum of a phalangiid. He finds it difficult to conceive how previous workers, especially Bertram, have missed this obvious fact.

The work throughout is clearly illustrated by a series of excellent line drawings and diagrams, many of which are original, so that this monograph is an admirable work on the comparative anatomy and function of the arachnid feeding apparatus. A copious bibliography is appended, many of the references being frequently cited in the text. E. D. H.

PROCEEDINGS OF THE SOCIETY

AN ORDINARY MEETING

OF THE SOCIETY AND AN EXHIBITION OF INDUSTRIAL MICROSCOPY WAS HELD IN THE
GREAT HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE,
LONDON, W.C.1, ON WEDNESDAY, OCTOBER 15TH, 1947, AT 5.30 P.M., DR. R. J.
LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed and signed by the
President.

New Fellows.—The following Candidates were balloted for and duly elected
as Ordinary Fellows of the Society:

A. Brecker.	London.
H. A. Burgess.	London.
F. Galloway.	Leicester.
S. R. Gupta.	Allahabad.
N. S. Macqueen.	Sevenoaks.
J. C. Meadley.	Manchester.
B. O. Payne.	Burnholme.
R. W. Weeks.	London.
G. I. Wells.	Harrogate.

Nomination Certificates in favour of the following candidates were read for the
first time and directed to be suspended in the Rooms of the Society in the usual
manner:

R. A. Beesley.	Oxford.
M. Bradshaw-Bond.	Gosport.
J. Cheswick.	Didcot.
J. W. Durden.	Gerards Cross.
A. Garth Sabell.	Birmingham.
F. L. Gavins.	Keighley.
H. S. Job.	Egypt.
R. Kirk.	Khartoum.
S. Rohatgi.	London.
S. E. Sorrell.	Forest Hill.
E. O. Strauss.	Warrington.
A. F. Stallwood.	London.
E. C. Vanessen.	London.
F. C. Wise.	London.
M. R. Young.	Croydon.

Donations were reported from:

Messrs. Arthur Barron, Ltd.—
" Petrographic Micro-Technique." By A. W. Weatherhead, F.R.M.S.

Mr. A. G. Batley—
" A Treatise on the Diatomaceæ." By H. Van Heurck (1896).

Messrs. Faber & Faber, Ltd.—
" The Microscope and the Practical Principles of Observation." By
Theodore Stephanides.

Brigadier H. Gilbertson Smith, *C.B.*, *O.B.E.*, *M.C.*, F.R.M.S.—
Cuff-type microscope by Dolland, *circa* 1780, complete with accessories,
including solar microscope and case.

Mr. J. Smith, F.R.M.S.—
The sum of £1 13*s.* 8*d.*

Messrs. Williams & Norgate, Ltd.—
" Illuminants and Illumination for Microscopical Work." By F. E. J.
Ockenden, M.I.E.E., F.Inst.P.

Messrs. Woodroffes (executors of the late George Tilling)—
Legacy of £200.

Mrs. Julian van Heurck—
" Levensschetz van Dr. H. Van Heurck en Overzicht van Zijn Verzame-
lingen."

Exhibition.—The reopening of the Industrial Section was introduced by a number
of demonstrations given by the following Research Organizations and Firms:

Messrs. R. & J. Beck, Ltd.
British Coal Utilization R.A.
British Cotton Industries R.A.
British Electrical & Allied Industries R.A.
British Food Manufacturing Industries R.A.
British Leather R.A.
British Non-Ferrous Metals R.A.
Messrs. W. Watson & Son.
British Pottery R.A.

British Scientific Instruments, R.A.
Messrs. Cooke, Troughton & Simms Ltd.
Messrs. Igranic Electrical Co. Ltd.
Messrs. Optical Measuring Tools Ltd.
Printing and Allied Trades R.A.
R.A. of British Paint, Colour and Varnish
Manufacturers.
Messrs. Tootal, Broadhurst & Lee, Co.

Further exhibits and demonstrations were made by:

Mr. F. C. Grigg, F.R.M.S., Hon. Secretary, on behalf of the Society.
Mr. H. Gunnery, F.R.M.S.
Mr. F. D. Armitage, F.L.S., F.R.P.S., F.R.M.S., Hon. Secretary of the Section
for Industrial Microscopy, described the exhibits and thanked the exhibitors
for their co-operation.

Announcement.—The President made the following announcement:

The next Meeting of the Biological Section will be held on Wednesday, November
5th, 1947.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, NOVEMBER 19TH, 1947, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the President.

New Fellows.—The following Candidates were balloted for and duly elected Ordinary Fellows of the Society:

R. A. Beesley.	Oxford.
M. Bradshaw-Bond.	Gosport.
J. Creswick.	Didcot.
J. W. Durden.	Gerards Cross.
A. Garth Sabell.	Birmingham.
F. L. Gavins.	Keighley.
H. S. Job.	Egypt.
R. Kirk.	Khartoum.
S. Rohatgi.	London.
S. E. Sorrell.	Forest Hill.
A. F. Stallwood.	London.
E. C. Vanessen.	London.
F. C. Wise.	London.
M. R. Young.	Croydon.

Nomination Certificates in favour of the following Candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

Honorary Fellowship—

J. E. Barnard.	Elected 1895.

Ordinary Fellowship—

W. Burrells.	Kenton.
W. J. Edwards.	New Malden.
H. R. Fleck.	Biggin Hill.
N. M. Hancox.	West Kirby.
C. L. Hare.	Liverpool.
T. W. Paddon.	Rochester.

Papers.—The following communications were read:

Electron Microscope Studies of Tissues from High- and Low-Breast Cancer Strains of Mice —

General Introduction—this was made by Professor R. D. Passey, *M.C.*, M.D., D.P.H.

A Description of Experiments and Results, by Dr. L. Dmochowski.

A Survey of Electron Microscope Technique, by Professor W. T. Astbury, M.A., Sc.D., F.R.S.

Followed by the Demonstration and Description of Electron Microscope Results made by Dr. R. Reed.

A very cordial vote of thanks was extended to all the Speakers.

Announcements.—The President made the following announcements:

The Industrial Section will meet in the Hastings Hall on Wednesday, November 26th, 1947, at 5.30 for 6.00 p.m.

The Biological Section will meet in the Hastings Hall on Wednesday, December 3rd, 1947, at 5.30 for 6.00 p.m.

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, DECEMBER 17TH, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the President.

New Fellows.—The following Candidates were balloted for and duly elected:

Honorary Fellowship—
J. E. Barnard.   Elected 1895.

Ordinary Fellowship—
W. Burrells.   Kenton.
W. J. Edwards.   New Malden.
H. R. Fleck.   Biggin Hill.
N. M. Hancox.   West Kirby.
C. L. Hare.   Liverpool.
T. W. Paddon.   Rochester.

Nomination Certificate in favour of the following Candidate was read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

L. R. Sadler.   Gidea Park.

Papers.—The following communications were read and films shown:

Mr. Sydney Gregory, B.Sc., Member of Council of the Scientific Film Association, described, *vice* Mr. Basil Wright, B.A., the Aims and Objects for which the Association was formed.

Films on " Tissue Culture by Phase-Contrast " and " Cleavage in the Sea Urchin Egg " were shown and described by Dr. A. F. W. Hughes, M.A., F.R.M.S.

The film by Dr. Adrianus Pijper, D.Sc., F.R.M.S., on the " Shape and Motility of Bacteria " was also shown and described.

A very cordial vote of thanks was extended to the Speakers.

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Announcements.—The President made the following announcements:

The Section for Industrial Microscopy will meet in the Hastings Hall on Wednesday, December 31st, 1947, at 5.30 for 6.00 p.m.

The Biological Section will meet in the Hastings Hall on Wednesday, January 7th, 1948, at 5.30 for 6.00 p.m.

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The Proceedings then terminated.

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VI.—THE PHASE-CONTRAST MICROSCOPE WITH PARTICULAR 535.824
REFERENCE TO VERTICAL INCIDENT ILLUMINATION.

By E. WILFRED TAYLOR, *C.B.E.*, F.Inst.P., F.R.M.S.

THIRTEEN PLATES AND ONE TEXT-FIGURE.

MANY laboratories are now equipped with means for studying the phase-contrast image of transparent objects and much work has been done in the investigation of living and inert material. This method of producing a contrasted image is not, however, confined to any particular wavelength of light and important results may be obtained beyond the visual region. With apparatus designed for the purpose it is possible to explore the infra-red and the ultra-violet regions of the spectrum and to record the results on the photographic plate. A fluorescent screen may also offer possibilities in the ultra-violet region.

Visual observation is not necessarily confined to transparent preparations, as any object which gives rise to specular reflection may also produce small phase differences in the reflected rays. As with transparent objects, the images formed may be of a positive character, depending on the characteristics of the phase plate. In use the method is very striking in its power to reveal minute irregularities on the surface of a carefully prepared specimen.

The normal phase-contrast instrument and the principles involved are already known (Taylor, 1946) and it is now proposed to describe a form of apparatus devised especially for the study of certain opaque materials which, after suitable treatment, give rise to specular reflection.

It is realized that similar apparatus has been described elsewhere and that phase-contrast photographs of metallic specimens have already been produced in this country (Cuckow, 1947, 1949) and in the United States (Jupnik *et al.*,

1946, 1948).   It will be clear, however, that parallel developments have been undertaken quite independently and that the object here is to describe a particular form of apparatus and to show some examples of the results obtained.

The Apparatus (fig. 1).

Light from a high-intensity lamp 1 is converged by the lamp condenser 2 to form an image of the light source approximately in the plane of the annulus 3. The light thereafter passes through the convergent lens 4 to suffer partial reflection at the cover-glass 5, after which it is converged by the objective 6 (which is assumed to be focused on the surface of the specimen) to form an image of the annulus at 7 somewhat above the focal plane of the objective.   After reflection at the surface of the specimen, the virtual image of the annulus lies below the focal plane at 8 and the rays proceed back through the objective and cover-glass to form a real image of the annulus in the plane of the phase plate 9 situated somewhat above the back focal plane of the objective.
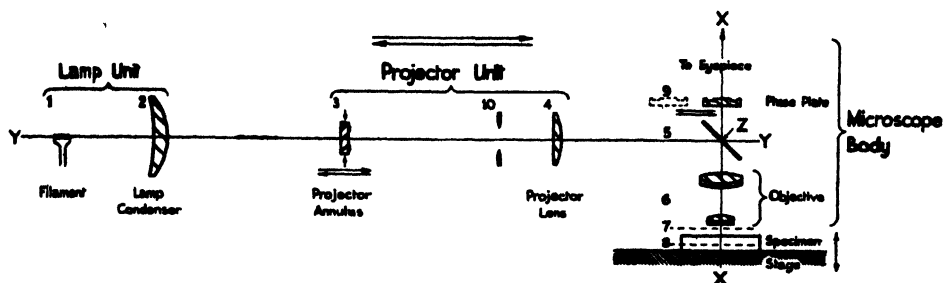
Fig. 1.—The optical system : phase-contrast and vertical incident illumination.

The objective 6 with its cover-glass reflector 5 and phase plate 9 is mounted in a " quick change " device, and is one of a series with initial magnifications of 10, 20, 40, and 95.   The phase plates integral with the various objectives are of annular form designed to produce phase differences of $\lambda/4$ and to absorb a proportion of the " direct rays."   Throughout the series a common ratio is preserved between the inner and outer diameters of the phase annuli corresponding to that of the annulus 3 ; thus by varying the magnification of the image of the latter a perfect match may be obtained with the phase plate of any one of the four objectives.

The function of what may be termed " the annulus projection unit " is twofold.   In the first place, it is required to project an image of the annulus 3 precisely into the plane of the phase plate 9, and in the second place this final image must have precisely the correct magnification in order that the two annuli may be brought to coincidence.   These requirements are met if the projection unit is made to move towards or away from the objective, and the annulus can also be moved axially towards or away from the lens 4.   In addition, the image of the annulus must be precisely coincident with that of the phase plate, and this is achieved by suitable centring adjustments applied to the annulus 3.

The cover-glass reflector 5 may be rotated about the optical axis of the microscope body XX and may be pivoted about a second axis Z at right angles thereto.   The axis of the annulus projector unit YY is also normal to XX.

The phase plate 9 may be moved out of the beam and a glass disc with an opaque annulus substituted, in which case all the direct rays proceeding to the focal plane are occulted and dark-ground effects are produced, or, alternatively, the annulus 3 may also be tripped out of position to enable normal incident illumination to be used.

As with the unit designed for use with transparent objects, an auxiliary microscope is substituted for the normal eyepiece while carrying out certain of the adjustments.

Had the phase plate been placed in the back focal plane of the objective, as in the normal phase-contrast instrument, unwanted and harmful light reflected from the surfaces of the phase plate would have been directed back into the field of the eyepiece to the detriment of image contrast ; in addition, the whole of the incident light would have passed through the phase plate, with consequent losses by absorption.

The Adjustments.

The selected objective, complete with cover-glass and phase plate, is clipped into position in the changer and a parallel-ended disc of stainless steel or other suitable substance with a plane polished upper surface producing specular reflection is placed on the stage. The high intensity lamp (which may utilize a filament or a high-pressure mercury source) is set up co-axially with the projection unit and focused on the annulus 3, which is then tripped out of position.

The objective is rotated to bring the cover-glass reflector into position and the latter is angularly adjusted until the field of view of the eyepiece is filled with light. The stage is then racked up or down until the upper surface of the specimen is brought into focus. In order to perfect these adjustments the iris diaphragm 10 in the projection unit is partly closed and the cover-glass rotated and pivoted until the image of the iris is central in the field of view. The iris is then opened to the minimum necessary and the cover-glass thereafter needs no further attention.

At this stage the eyepiece is removed and the auxiliary microscope inserted in its place and focused on the phase plate.

The annulus is now tripped into position and the whole projection unit is racked towards or away from the objective until the image of the annulus is seen sharply in focus in the field of the auxiliary microscope. The centring screws in the projection unit are now manipulated until the two annuli are concentric with one another. If the image of the annulus is larger in diameter than the phase annulus, the distance between the former and the lens 4 must be increased and the unit refocused until finally the two are in coincidence. The auxiliary microscope is now withdrawn and the eyepiece replaced.

The stainless steel disc is removed and a polished and etched metallurgical specimen substituted and approximately focused by readjustment of the stage. On substituting the auxiliary microscope it may be seen that the two annuli are no longer coincident—in which case recourse must be had to the centring screws —or that the image of the annulus is vague and nebulous. The former merely indicates that the surface of the specimen is not quite normal to the axis of the

microscope, which implies re-centring, and the second that the surface diffuses too much light to give promise of a good phase-contrast image.

If the conditions are found to be normal the auxiliary microscope is withdrawn, the eyepiece replaced and the specimen carefully focused, using the fine motion adjustment.

On the substitution of an opaque annulus in place of the phase plate, the image will be seen by dark-ground illumination, or on replacing the phase plate and tripping out the annulus, vertical incident illumination will result.

Although somewhat tedious to describe, the necessary adjustments are easily and quickly made. The instrument is shown in fig. 2.

Interpretation of the Image.

The correct interpretation of all that is seen in the phase-contrast image can only result from experience. Crystal boundaries are very clearly revealed, and as both the polishing and etching processes are affected by the orientation of the individual crystals in a mosaic, slight differences in the general level are produced. Such differences give rise to slight changes in the phase of the reflected light and produce areas of significant contrast gradations in the image. It is also possible that changes in phase may result from slight but differential penetration of the rays into the surface of the specimen. It is known that the method will assist in the identification of intermediate transformation products which are otherwise difficult to distinguish (Cuckow, 1947).

Surface irregularities are very clearly revealed, and in some cases details too small to be resolved in the normal manner give rise to sufficient change in the wave front to produce clearly recognizable features in the phase-contrast image.

The method is new and appears to open interesting future possibilities for the metallurgist and mineralogist.

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TAYLOR, E. W. (1946).—J. R. micr. Soc., **66**, 1–8.
CUCKOW, F. W. (1947).—Nature, Lond. **159**, 639.
CUCKOW, F. W. (1949).—J. Iron Steel Inst., **161** (1), 1–10.
JUPNIK, H., OSTERBERG, H., and PRIDE, G. E. (1946, 1948).—J. Opt. Soc. Amer., **36**, 710A ; **38**, 338.

DESCRIPTIONS OF PLATES.

PLATES I AND II.
White-heart malleable iron (× 750) : I. Vertical incident light ; II. Phase-contrast.

PLATES III AND IV.
Grey iron (× 1800) : III. Vertical incident light ; IV. Phase-contrast.

PLATES V AND VI.
High-speed tool steel (× 750) : V. Vertical incident light ; VI. Phase-contrast.

PLATES VII AND VIII.
Case-hardened mild steel (× 750) : VII. Vertical incident light ; VIII. Phase-contrast.

PLATES IX AND X.
Zinc (× 150) : IX. Vertical incident light ; X. Phase-contrast.

PLATES XI AND XII.
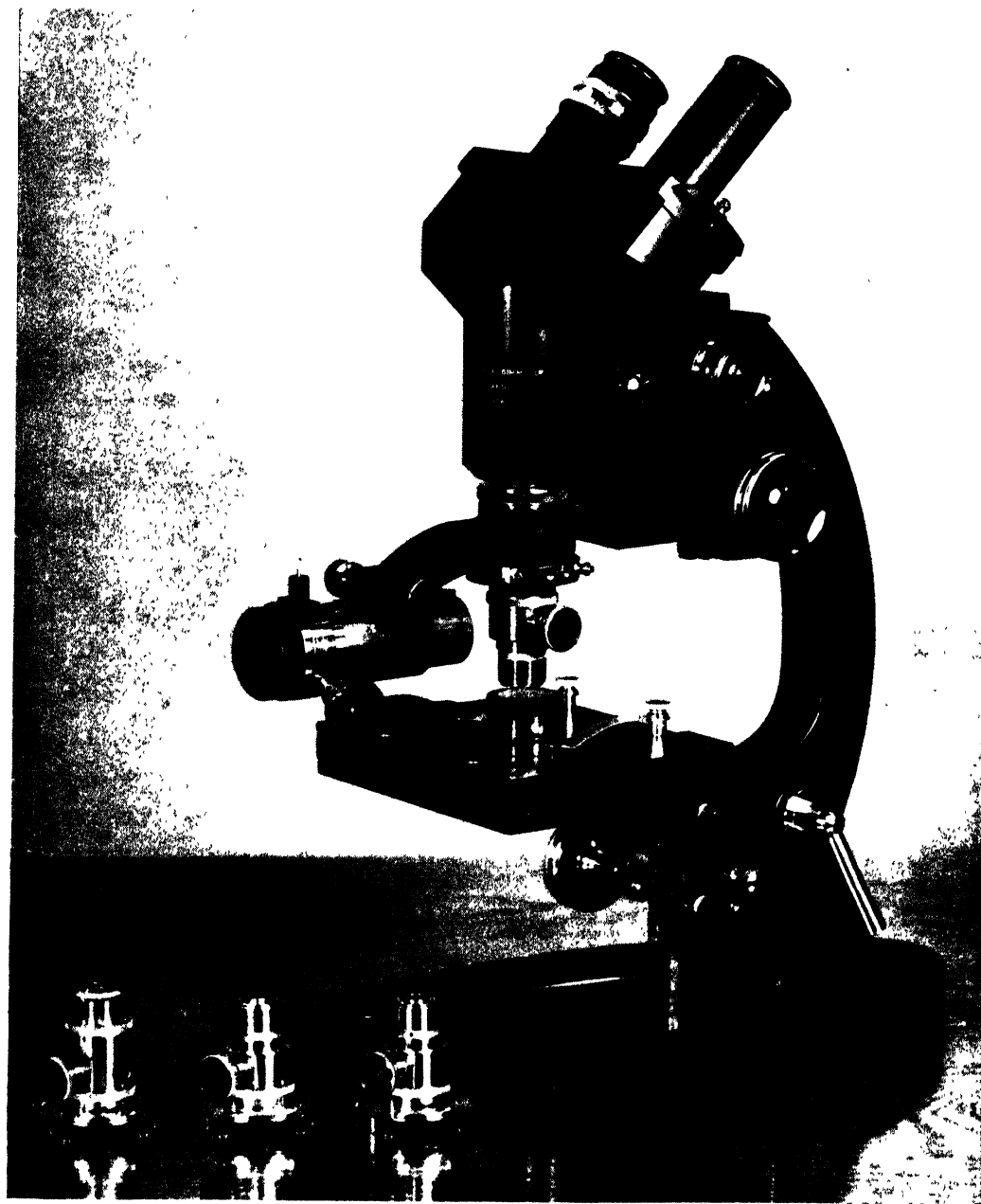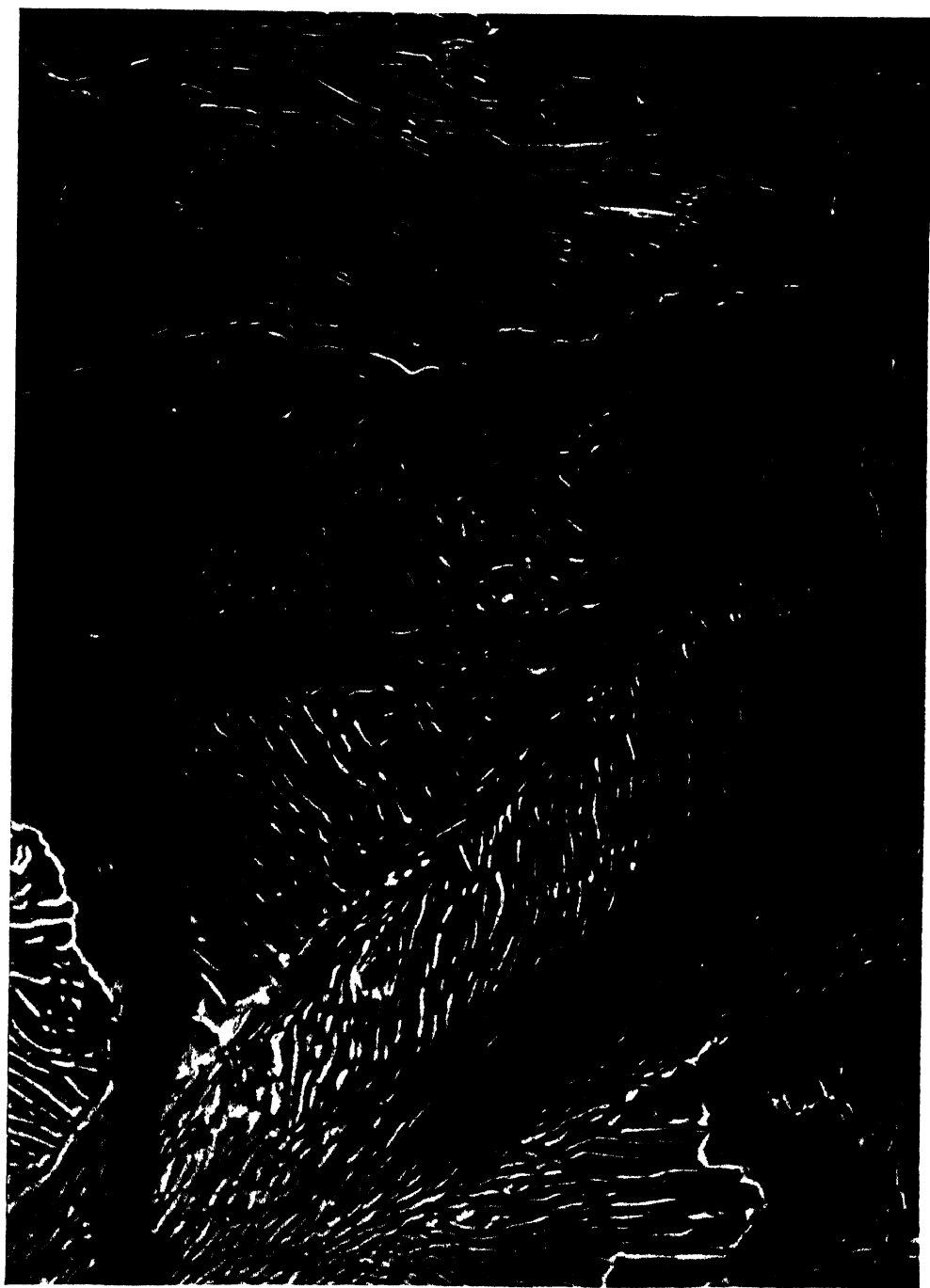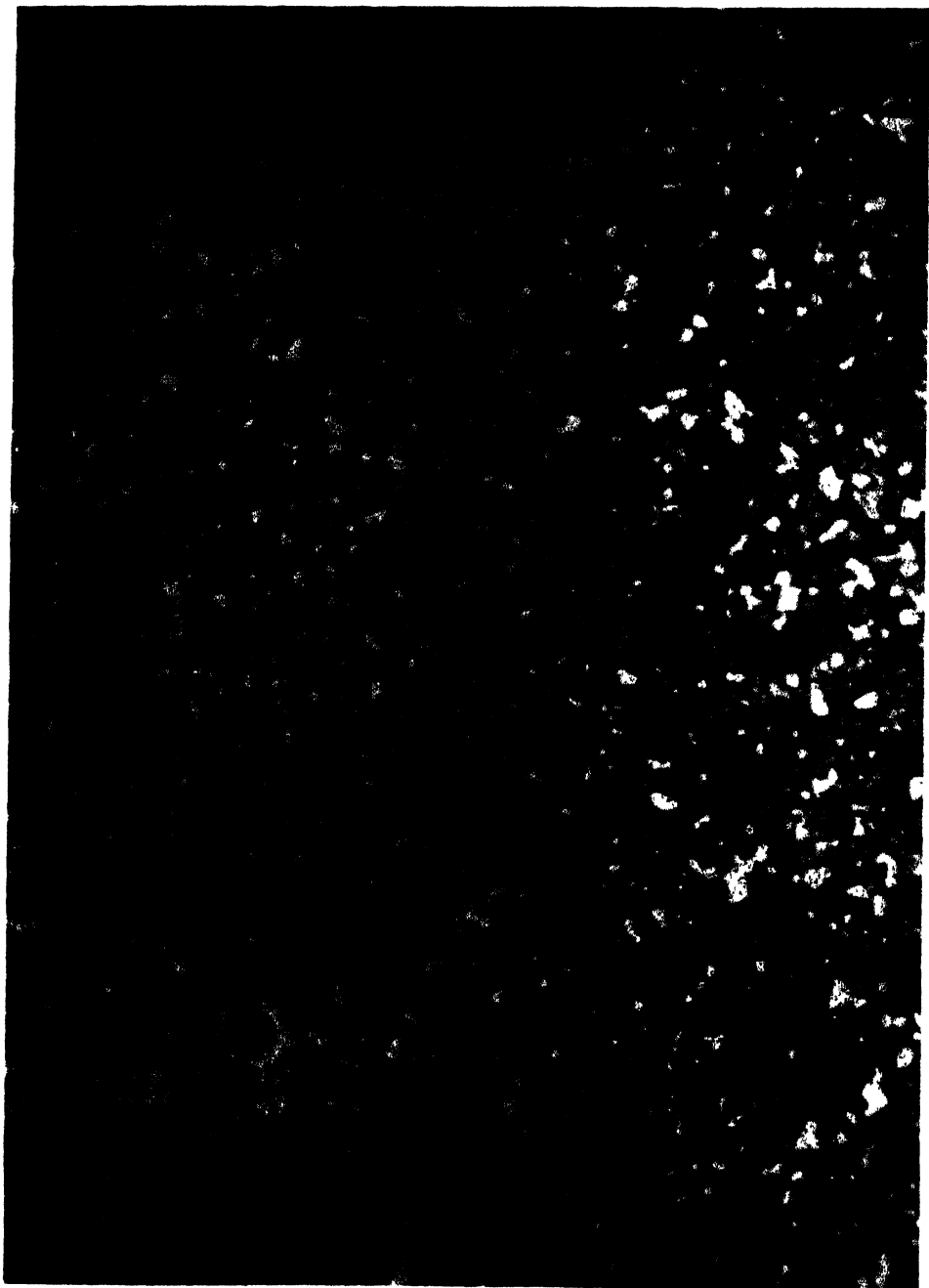Aluminium (× 350) : XI. Vertical incident light ; XII. Phase-contrast.

Fig. 2.—The phase-contrast microscope for use with vertical incident illumination.
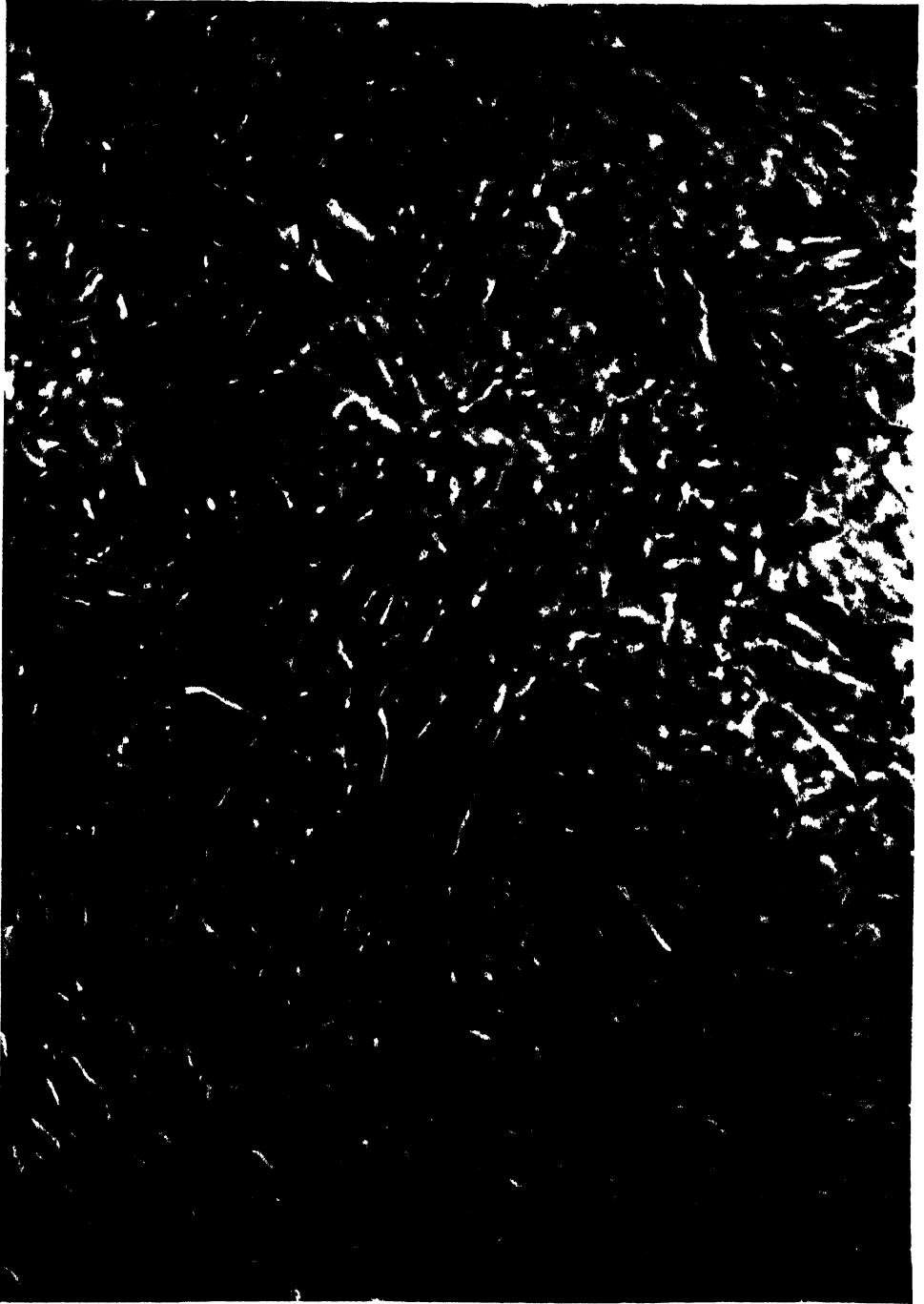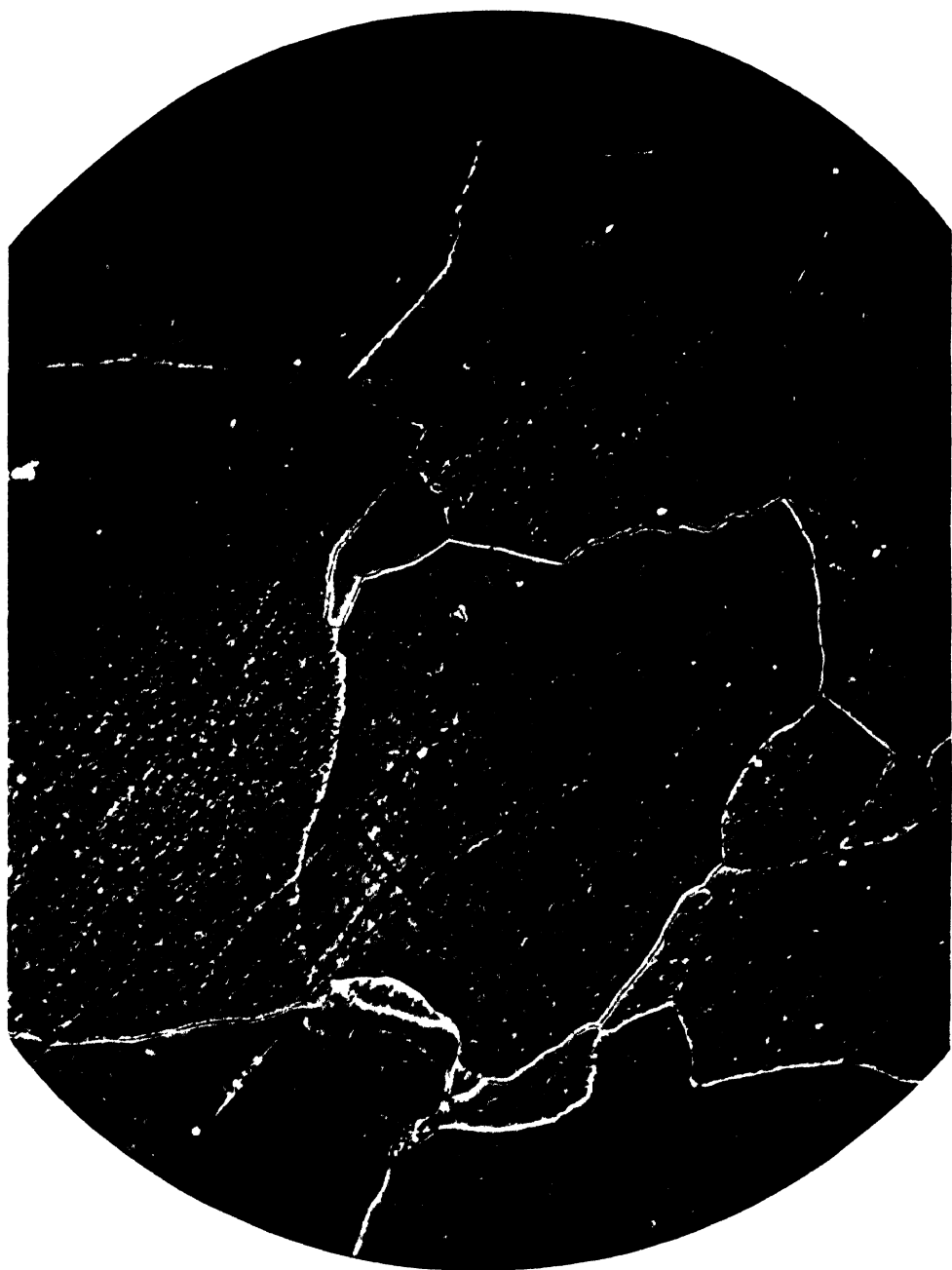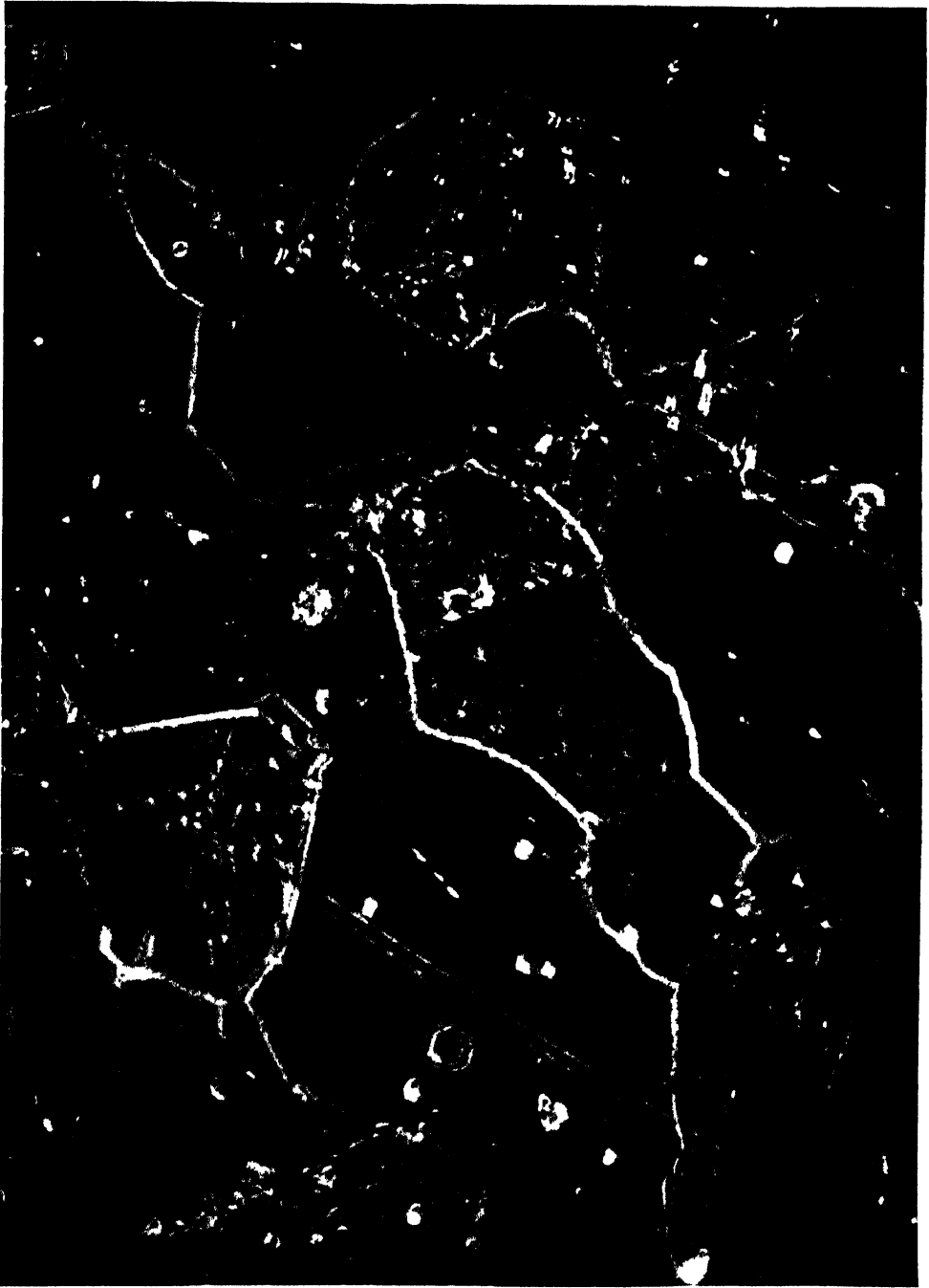
VII.—THE TECHNIQUE OF CINÉ-PHOTOMICROGRAPHY OF LIVING CELLS.

By A. F. W. Hughes, M.A., Ph.D., F.R.M.S.

(Sir Halley Stewart Research Fellow, Strangeways Research Laboratory.)

Two Plates and Three Text-Figures.

Introduction.

The purpose of this paper is to describe the technical details of the methods used in research on living cells by phase-contrast ciné-photomicrography.

The cells studied in this way are in the outgrowth of tissue cultures of embryonic or young tissues of vertebrates, such as Amphibia, the chick, and the mouse.

The combination of tissue culture with phase-contrast microscopy is of great value. The thin, flat cells in the outgrowth of suitable cultures are beautiful objects for such study. Cells can be followed through the mitotic cycle and early stages of prophase can be recognized. Within the cell there is constant movement, which invites study as a means of approaching the problems of the nature of living cytoplasm.

The dynamic aspect of the living cells which is thus presented to the observer demands the use of the ciné-camera. The movements which are seen cannot adequately be perceived in direct observation, due both to their complexity and to the length of the time-scale over which they take place.

Phase-Contrast Microscopy of Tissue Cultures.

The phase-contrast method itself has already been the subject of a number of papers (references in Taylor, 1946) and requires no further description. Its use with tissue cultures has already been briefly described (Hughes and Swann, 1948, Hughes and Fell, 1948).

The tissue cultures used are " hanging-drop " preparations, with a free air surface below the culture. This air gap between object and condenser is no great impediment to the use of the phase-contrast microscope. Certain precautions must be observed, however :

(1) The total thickness of the preparation has to be kept as small as possible. The working distance of a condenser is less across air than through glass. The culture chamber which we use consists of a brass ring about 1 mm. in thickness to which two cover-slips are sealed. On the upper cover-slip the culture is mounted ; the lower is plain, and seals the chamber.

(2) The condensation of moisture on the inner surface of the lower cover-slip must be prevented during observation ; this can be effected in warm stage work by enclosing the microscope in a box in which the bottom is at a higher temperature than the top. The lower surface of the culture chamber will thus be slightly warmer than the upper. The " hot-box " used for this purpose will be later described.

(3) The air-culture medium interface is not a plane surface, and may distort the image of the illuminating annulus, formed in the plane of the phase-plate of the objective. Loss of image quality can result in this way.

The difficulty is largely overcome by the use of a method developed by Dr. H. B. Fell, and briefly described by Hughes and Fell (1948). Part of the plasma coagulum next to the explant is removed. This gap is filled by fluid which exudes from the rest of the clot. Cells grow out from the explant into this area as a thin layer, flat against the cover-slip. These cells thus grow in a fluid medium, in which the outgrowth is nearly as profuse as that into the coagulum. The cells in the fluid phase are thinner, and a more accurate image of the condenser annulus is formed in this area.

Photographic Technique.

The objectives and condensers used in the phase-contrast microscope which is here described have been manufactured by Messrs. Cooke, Troughton & Simms Ltd.

The first set of these to be used was the standard equipment, now widely known (Taylor, 1946). Mainly with the use of the ×95 objective, a series of photographic tests was made with a number of emulsions on 16-mm. film, with the co-operation of the research staff of Messrs. Kodak Ltd.

The main object of these tests was to gain increased contrast. In pl. I are shown enlargements on three different film stocks, of a single object, a cell in tissue culture.

It was soon realized that fineness of grain as well as increase in contrast contributes to improvement in the photographic quality of the result. These two factors were not studied separately because, in general, these features tend to be associated. However, we may here refer to each in turn.

Such was the need for contrast in this work that the film finally chosen for use in 16-mm. work was that with the highest available gamma. It is a Kodak emulsion known as " film for ciné-photomicrography " and has been extensively used during the past year for research in cell division.

The same degree of contrast could, no doubt, have been obtained in these photomicrographs by varying the characteristics of the objective—for instance, by decreasing the transmission of the undeviated light through the phase ring of the objective. However, to gain extra contrast *via* the emulsion means that resolution also is increased, because of the fineness of grain of the high-contrast film.

Resolution of photographic emulsions is usually expressed by the maximum number of lines per millimetre which it would be possible to resolve, for instance,

if a system of black lines on a white surface were photographed. With any other subject this degree of resolution is never obtained, but this optimum figure serves as a convenient index.

In photomicrography, the following factors affect resolution : the grain of the film, the magnification, and the resolution of the objective. We can link together the first two factors by calculating the maximum number of lines on the film which correspond to 1 micron of the object.

In pl. I (figs. 1–3) are reproduced three photomicrographs of the same object, taken on three different emulsions. The data relative to the negatives of this picture are given in Table I.

TABLE I.—DATA CONCERNING THE NEGATIVES OF THE THREE PHOTOMICROGRAPHS
(pl. II, figs. 1–3).

Film.	Gauge.	Lines per mm.	Gamma.	Fig. No. in pl. II.	Mag. on film.	Lines per micron of object.
R.55	16 mm.	40–50	1·5	1	200	8–10
C.-P.-M.	16 mm.	90	>5	2	200	18
1372	35 mm.	150	3	3	700	100

The progressive improvement in these three is largely due to increase in the value of the lines per object-micron index. However, between Ia and Ib there is also an increase in contrast, and for the last picture a higher initial magnification and a different ×95 objective were used.

This objective was specially prepared by Mr. E. W. Taylor, of Messrs. Cooke, Troughton & Simms Ltd. It is corrected for blue light of wave-length 4600 Å, and its purpose was to make possible the use of certain fine-grain emulsions which are blue-sensitive only. It was found to have intrinsic advantages in contrast and resolution.

Phase-contrast objectives should be used as virtual monochromats, at the wavelength for which the retardation of the phase plate is $\frac{1}{4} \lambda$. The gain in resolution with the standard objective by using a green filter is considerable. A phase-contrast objective has almost no depth of focus and shows very clearly the change of focus with wavelength characteristic of an achromat.

In the blue-corrected objective, the maximum correction for both spherical and chromatic aberration are made to coincide at 4600 Å. The shorter wavelength may also contribute to the increased resolution.

For 35-mm. work this objective is used with a blue-sensitive film of very fine grain, primarily used for sound recording. It is emulsion No. 1372 in the Kodak range.

An excellent test for the resolution of a phase-contrast microscope is the following. On the surface of the sloughed epithelial cells that occur plentifully in saliva is a system of fine lines which are about 0·3–0·7 microns apart. Not every epithelial cell shows them, and they are only clearly seen under optimum conditions. Pl. I, fig. 4, shows how they are resolved by the special ×95 objective at 4600 Å.

An ordinary oil-immersion object of N.A. 1·3, used with a dry condenser,

should be able to resolve about 10 lines to the micron, according to the ordinary Abbé relationship. Yet there is a marked improvement in phase-contrast photographs, in which the lines/object micron index is increased from 20 to 100. This apparent disproportion is due not only to the fact that the latter index is a theoretical maximum. The resolution of the phase microscope exceeds that given by the Abbé relationship. This fact, which is well known to phase-contrast microscopists, is gratifying, although as yet unexplained.

The light source which is used is a high-pressure mercury arc, the well-known 250-watt " Mercra " lamp of Messrs. B.T.H. The characteristics of such sources have recently been described by Bourne (1948). The 250-watt lamp should be allowed to run for at least $\frac{1}{2}$ hour before photography is begun, otherwise the first few feet of film will be seriously underexposed, if the normal setting of filters and rate of exposure are used.

The residual ultra violet that is passed by the glass window of the lamp is absorbed by a Wratten filter 2 A, and a piece of Chance glass, O.N. 19, is used as a heat absorbent. The green lines are isolated by a Wratten filter No. 60 and the blue by a No. 47 with a transmission of 40 p.c. at 4600 Å. For film which is blue-sensitive only, one can use instead a Wratten No. 32, which transmits 70 p.c. at 4600 Å. and also a wide band in the red, which will not affect the film.

It is thus possible to vary the contrast and resolution obtained in ciné-photomicrography within fairly wide limits, by choosing different emulsions on sub-standard and standard gauges, used at different magnifications. However, every gain in photographic quality needs a greater intensity of light on the film, transmitted through the living object on the microscope. The limit to this improvement of the result is set by the amount of light which the living material will tolerate without disturbance of the biological functions that are being studied.

It is convenient to use the same intensity of light for the photographic exposures as for the occasional observation between exposures. Some standard and reproducible means of controlling the light intensity is desirable. I use a set of neutral filters, mounted between two discs about 8 inches in diameter, in which five circular holes, $1\frac{3}{4}$ inches diameter, have been made. One of these has no filter, and through successive apertures the light intensity is reduced by respectively $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ and $\frac{1}{16}$ the full value (pl. II, fig. 6). By this means, also, a series of graded exposures can be given, from which the film gamma can be deduced by measurement of the densities of the resulting negatives.

Again, the light tolerance of the material depends on the length of time for which it is desired to photograph a single preparation. Thus each type of material needs preliminary study with respect to its light sensitivity and the degree of contrast and resolution required in the final film.

In recording cell division in cultures of chick and mouse tissues at high magnification, I use the Kodak high-contrast 16-mm. film for ciné-micrography with the ×95 objective, and an initial magnification on the film of about 200. Half-second exposures are made at intervals of 2–5 seconds, and a cell is followed through division for a period of 1 hour or more. The illumination of the object is interrupted between exposures, except when the focus of the microscope is

being checked. Under these conditions it is unusual for a cell to show symptoms of being adversely affected by the light, as for example, by a prolongation of metaphase or a disturbed cleavage. However, it is not advisable to attempt to follow successive mitoses in the same culture.

By using a film with a lower contrast, such as Super-X, or with a much less fine grain, such as R55, the exposure is cut down by a factor of 5 times or more. On the other hand, if one wishes to photograph the same sized field in the object on 35-mm. film, then the magnification must be increased by a factor of 2·5 and the intensity of illumination by the square of this, namely approximately 6 times. At 4600 Å. the 35-mm. film 1372 has about one-third the sensitivity of the 16-mm. film for ciné-photomicrography. Consequently, with the same size of image field, nearly 20 times as much light is needed for 35-mm. work as for 16-mm. recording, under these two sets of conditions.

For this reason I have worked mostly with the sub-standard gauge of film for high-power photography, where the field includes one single cell. Such needs to be supplemented, if possible, by photography on 35-mm. film. Single exposures with a Leica-size still camera of a reflex type are of great value, in order to obtain sets of stills of maximal quality.

Where, however, the need is for size of object field rather than magnification, then obviously 35-mm. film is preferable. At the Strangeways Laboratory the 35-mm. apparatus of the late R. G. Canti has been installed and is being used mainly for low-power work with tissue cultures. With a ×10 phase contrast objective, an object field of 0·37×0·47 mm. is photographed, at intervals of 1–2½ minutes. It is possible to follow cells under these conditions continuously for periods of 1 day or more, sometimes through two or more successive mitoses. A document-copying emulsion is used, such as Kodak " Microfile."

The choice between the two sizes of film depends upon the light sensitivity of the living material to be photographed and the nature of the emulsions available in each gauge. No general statement can be made ; 16-mm. work has the great advantage that the apparatus need not be built by engineers, and the Kodak film for ciné-photomicrography, available only in this gauge, is outstandingly suitable for this type of work.

A convenient size of object field comfortably to include a single fibroblastic cell in tissue culture is of the order of 30 × 50 microns. The requisite magnification on a 16-mm. frame is about 200. As the initial magnification of the objective is ×95, eyepieces are not usually less than ×4 ; the required magnification is most conveniently obtained by allowing the objective to form an image at a distance greater than 160 mm. and interposing a diverging corrector lens, so placed that the normal working distance between objective and object plane is not altered.

THE APPARATUS.

The general arrangement of the microscope and 16-mm. camera unit which I use is illustrated in pl. II, figs. 1–7. Its design has been largely determined by the equipment available which could be adapted for this purpose.

One of the most important features is that two alternative arrangements for viewing the image should be provided : one through an eyepiece of low

5

power in the ordinary way, the other through an optical system attached to the camera, which shows the size of field which is being photographed.   Only a small central area of the whole microscope field will be projected on to the film ; consequently, the restricted image of the camera viewer would not be suitable for searching for a part of the preparation suitable for photography.   It is highly desirable to restrict the illumination of the object to the area in the field of the camera, by focusing the field stop in the object plane by the ordinary Kohler method.

In my apparatus, the sliding arrangement of the Beck angular microscope is used for changing from an inclined visual eyepiece to a vertical projection system for photography (pl. II, fig. 3).   The vertical eyepiece tube contains the above-mentioned corrector lens.   The camera unit, carried on an external pillar, is
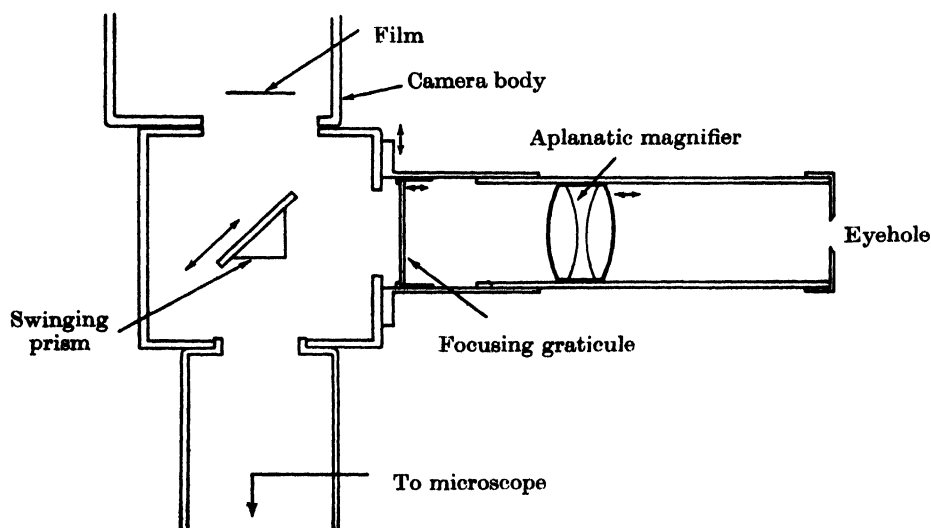

Fig. 1.—Vertical Section through focusing unit attached to camera.   (Not to scale.)

swung into position so that the optical axes of camera and microscope coincide. The viewer is virtually a reflex camera system, with a reflector which moves in and out of the optical axis between exposures (pl. II, figs. 1 and 3 ; text-fig. 1). It is more usual in such apparatus to employ a fixed semi-reflector or " beam-splitter " which divides the light between viewing eyepiece and film.   If the light is equally divided between the two paths, more than double the light intensity through the microscope is needed, for there is some absorption at the semi-reflecting surface.   It is possible, however, to deflect only a small fraction of the light, some 10 p.c. or so.   This system is suitable where a high intensity of light is used with a short exposure, as in normal speed ciné-photography.

In time-lapse work, with exposures of the order of a second and light intensities of the same level as are used in visual observation, in my opinion, the moving mirror system is to be preferred.

The viewing system of the camera unit has a right-angled prism mounted on a swinging arm, and worked through a lever by a cam on a 1-frame-per-turn shaft of the driving gear.   The general arrangement is indicated in text-fig. 1.
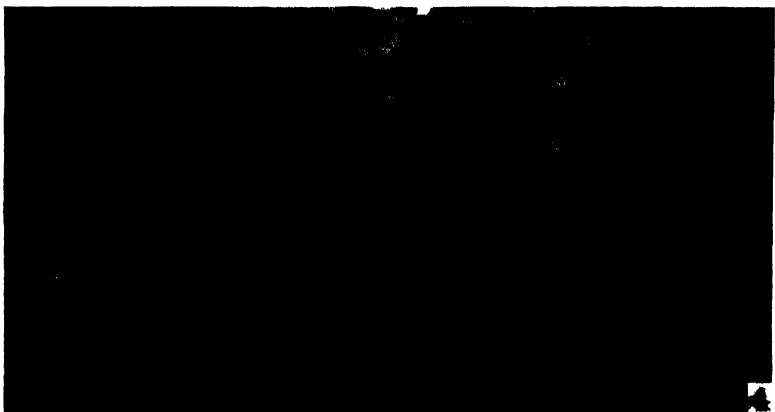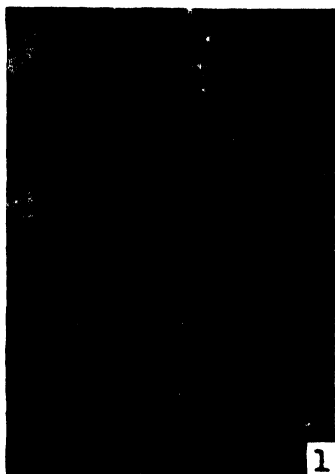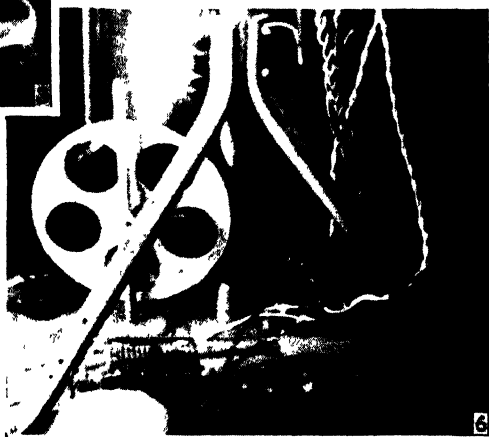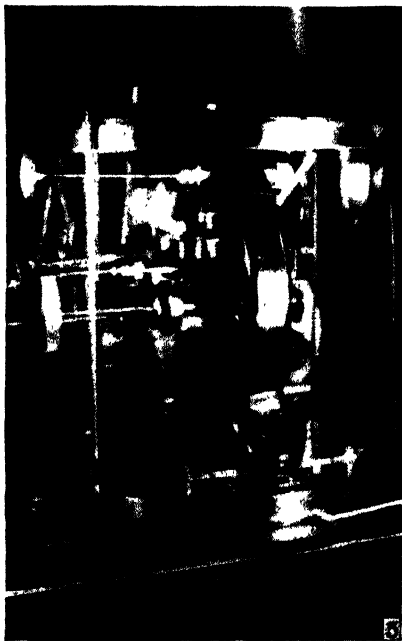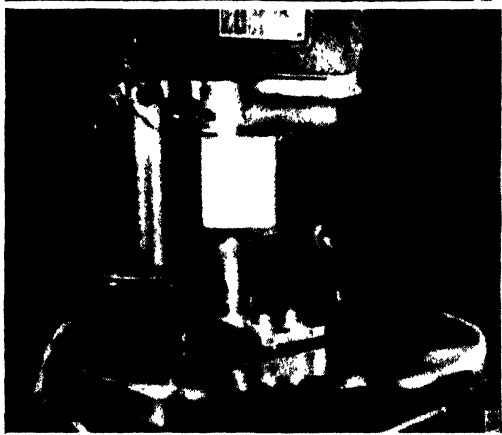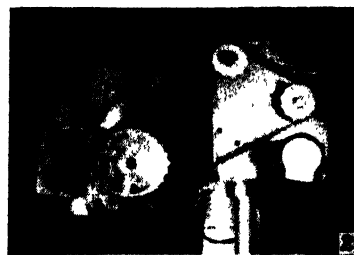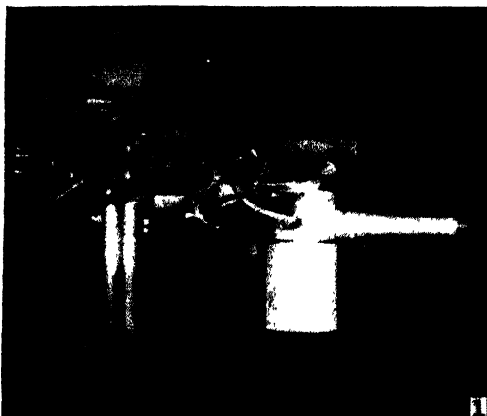
A graticule, on which the limits of the 16-mm. frame are marked, should be in the plane of the microscope image when deflected by the prism. This should be adjustable in all three dimensions to ensure that the images through viewing system and film gate accurately coincide, both in field and focus. The lateral image is viewed either with an aplanatic magnifier or a positive eyepiece.

Probably any ordinary 16-mm. camera could be adapted for this work, when combined with a viewing system such as that just described, in place of the camera lens. An " Ensign " camera is used in this apparatus and is built into a unit which carries the driving motor. This unit swings on a vertical pillar, as mentioned above; it can be removed, and is interchangeable with a " still " camera whose mountings fit on the same pillar.

The necessary modifications to the Ensign camera were as follows :

(1) The clockwork drive was removed and extensions were fitted to two shafts of the drawing mechanism ; one was the old governor shaft, through which the new external drive was arranged, the other a 1-frame-per-turn shaft from the gate mechanism, the function of which will be described below.

(2) New film sprockets were fitted with a single set of teeth on one side to take the single-perforated film for ciné-photomicrography.

The camera is driven by means of a synchronous gramophone motor, which fulfils these requirements :

(1) It should self-start, and assume its full running speed in as short an interval as possible (say $\frac{1}{4}$ second, or less).

(2) The running speed should be constant.

(3) It should come to rest as soon as possible after the circuit is broken.

(4) The vibration during running should be very small.

The motor is mounted on coil springs and the drive is taken through a chain and sprockets, with a spring-loaded jockey sprocket (pl. II, fig. 2).

There are several ways in which a motor can be arranged to drive a ciné-camera :

(1) Continuously, with the motor rotating steadily. The time of exposure, with most cameras, is nearly half the interval between successive exposures.

(2) Intermittently, the camera mechanism coming to rest between exposures, with the shutter closed.

(3) Again intermittently, with the camera mechanism coming to rest also during the exposure, with the shutter open.

In (1) and (2) the motor speed determines the time of exposure. A master controller in (2) repeatedly closes the motor circuit, at the intervals between successive exposures. In (3), this controller also governs the length of exposure.

(1) Is suitable for short and frequent exposures, (2) for longer intervals, and (3) for long intervals and long exposures. This system, for instance, may be necessary when the biological polarizing microscope is used (Hughes and Swann, 1948).

In phase-contrast work the camera unit is operated either on system (1). taking one picture per second with an exposure of $\frac{1}{2}$ second, or on system (2), with intervals between exposures of 2–6 seconds. If the driving motor fulfils the above conditions, it should be possible to change from system (1) to (2) and to keep the exposure constant.

For system (1) no mechanism other than the camera drive is required. For interrupted working, however, a timing mechanism is necessary to ensure that the camera comes to rest either with the shutter shut, as in (2), or open, as in (3).

The simplest form of timing mechanism suitable for a 16-mm. camera operating under the conditions just described is, in my experience, the following :

The motor circuit is controlled by two switches in parallel, either of which can complete the circuit (text-fig. 2). One of these switches (A) is actuated by the master timing control, the other (B) is on the camera. The master switch closes for a period shorter than that of the camera cycle. In system (2) the cycle begins with the camera at rest, with shutter closed and both switches open. The master switch closes, and as soon as the camera motor is turning, the second switch closes also. Then the master switch opens, but the camera drive continues until one frame has been exposed, and the camera switch is turned off when the shutter is again closed. A switch is provided to change from continuous operation (system 1) to interrupted working (system 2).

Several forms of master control can be used for intermittent operation. One is illustrated in pl. 2, fig. 7. A mercury switch is carried on a rocking arm. which is actuated by a camshaft rotating at constant speed. This arm can traverse over a bank of cams, which provide a series of intervals between exposures. In the first cam one gap is cut, and the interval given is that of the time of revolution of the shaft. Other cams have two and more gaps as desired.

Mercury switches are convenient for this purpose, but tend to work irregularly when closing a circuit for intervals less than 1 second, for the mercury bead within " bounces " when moved rapidly. An interval-timer giving a continuous variable interval from 2–60 seconds has for some time past been available at aircraft disposals stores in London. Here an arm, driven through an escapement mechanism, sweeps through an angle which is pre-set by a knob. and closes a circuit at one end of its traverse. At present, I am using an electronic timer made by Messrs. Sanders, Ltd., of Stevenage, which closes a circuit for $\frac{1}{2}$ second at intervals between $2\frac{1}{2}$ and 60 seconds.

The second switch (B), in parallel with the master, is carried on the camera unit. It is a mercury switch on a rocking arm, and is worked by a cam on the 1-frame-per-turn shaft mentioned above. This shaft also operates a revolution counter to record the number of frames exposed, and the same cam actuates the rocking arm which operates the prism arm of the camera viewer (pl. II, fig. 1).

This simple system of operation I have found satisfactory for interrupted operation with a 16-mm. camera. With a 35-mm. apparatus under the same conditions, the momentum of the driving mechanism is much greater and simple interruption of the motor circuit is not sufficient to bring the mechanism to rest in the right position and to give uniform exposures. A positive stop worked by another mechanism must be introduced.

It is highly desirable that there should be some means of interrupting the illumination of the preparation between exposures. This can be done electrically, either by switching on the light source (if a tungsten lamp) through the same circuit which controls the camera motor, or if the light source is a mercury arc which cannot be switched on and off, by means of an electro-magnetic shutter (pl. II, fig. 4).
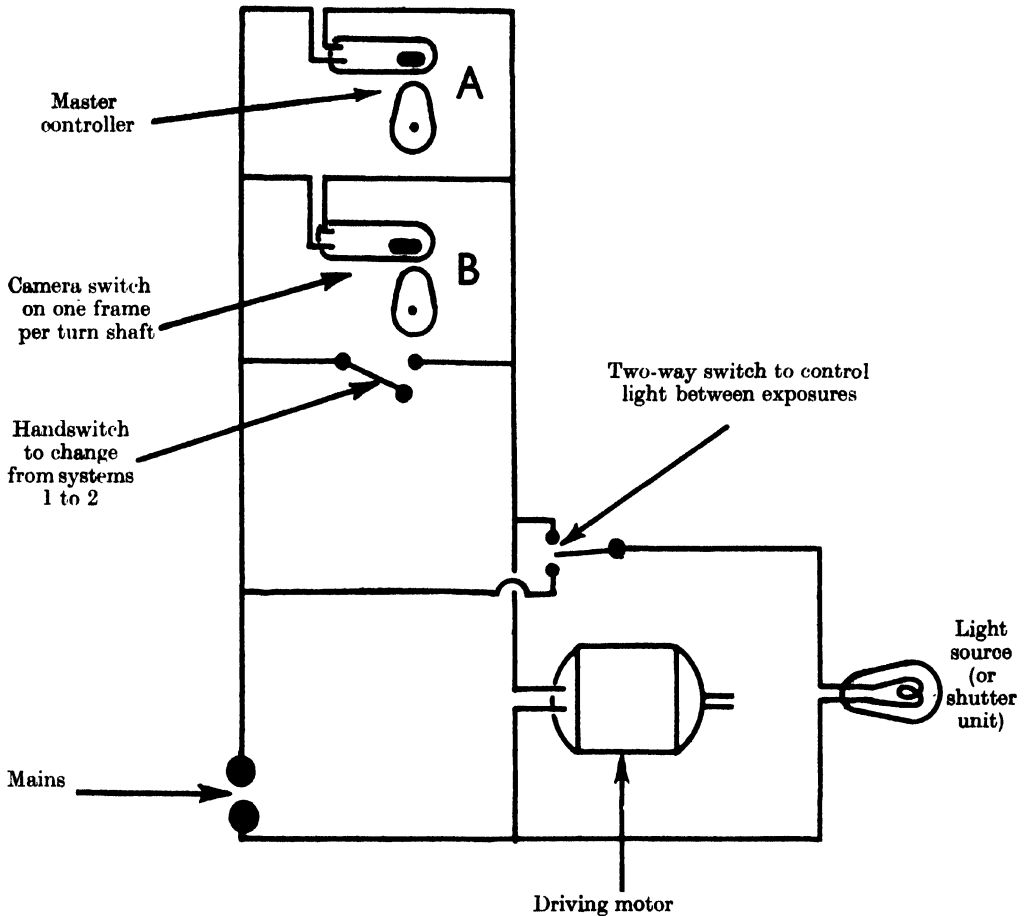

Fig. 2.—Circuit Diagram of Apparatus.

A two-way switch enables one to maintain illumination between exposures when necessary for adjustment of the microscope. The full circuit of the system is given in text-fig. 2.

Ciné-photomicrographic apparatus is generally built with elaborate precautions against the effects of vibration. Camera and microscope are usually insulated from each other, and often a very solid foundation is provided. Probably with 35 mm. cameras, and always with normal speed work, insulation of this kind is necessary. A spring mounting of the driving motor of the camera has proved sufficient, however, with this apparatus, which stands on an ordinary laboratory bench and is mounted on rubber bungs.

A sensitive test for mechanical vibration is to touch a part of the apparatus with the teeth (if one's own). A perceptible, though not uncomfortable, vibration is permissible in the camera. In the microscope there should be none. The physical principles of insulation from vibration are well described in Kodak Data Sheet A36.

Next, the arrangements for warm-stage microscopy may be described (pl. II, fig. 5, text-fig. 3). The microscope is mounted on a heating table and is enclosed in a cylindrical box of transparent plastic, about $\frac{1}{16}$ inch in thickness. This consists of two semi-cylinders, one of which can be rotated round the other, which is fixed. The box can thus be widely opened when necessary, but is kept closed during observation. All the controls of the microscope are extended to the outside of the box. These external controls end in large knobs, to increase the effective fineness of the adjustments of the microscope.

Under the microscope table are fitted heating elements with a resistance of 200 ohms. Two or more electric soldering iron elements in series are suitable. The circuit is controlled by a " Sun Vic " regulator, Type TS1.

This arrangement results in a temperature gradient through the box from bottom to top which serves to keep condensation off the lower surface of the culture chamber, as mentioned above. The spiral of the temperature regulator and the bulb of an indicating thermometer must, of course, be mounted at stage level.

This arrangement keeps the temperature of the stage constant to within $0 \cdot 3°$ C. Variation in temperature alters focus, for the air within the culture chamber obeys Charles' Law, and can flex the cover-slip on which the culture is mounted. A closer control of temperature can be obtained with the more sensitive regulator Type TS3, which can only be used with a relay, such as the hot-wire vacuum switch of Messrs. Sun-Vic Controls. This finer degree of control is necessary for following the growth of a culture over long periods with long intervals between exposures, and where the apparatus is left unattended, with infrequent adjustment of focus.

This " hot-box " is built up from two sets of special brass rings, separated by distance rods. These were made for me by my father, Mr. W. R. Hughes. A radial section through the upper set of rings is given in text-fig. 3.

THE PROCESSING OF THE FILM.

It is highly desirable to develop each reel of film as it is used in order to ensure that all is in order with the apparatus and its adjustment. To continue " shooting " without reasonable certainty of this, is to run an unnecessary risk of spoiling film.

Commercially, ciné film is processed by continuous methods in elaborate plant. Tank development of 35-mm. film in lengths of 100 feet may be done in a device which is marketed by Messrs. Kodak ; 16-mm. film can be handled in lengths of 30 feet or so by winding on a suitable frame, which can be immersed in a tank for the processing of radiographs.

A suitable frame can be made by bending ordinary 8-mm. laboratory glass

tubing into a rectangle. The film is wound on this in total darkness, an operation which needs some practice. It is anchored at each end by hooks of platinum wire. The adjacent sections of the film are separated by rubber spacing washers, which can be punched out of a car inner tube.

I use Kodak D196d for 3 minutes at 65° F., for developing the film for ciné-photomicrography. This is not necessarily the best developer, although I understand that little further improvement results from the use of fine-grain developers with emulsion of intrinsically fine grain. This film has a very adherent black backing which is only partially removed during developing and fixing. It persists particularly where the film touches the frame on which it is wound. I remove as much as possible of this backing when the film is wet by running a finger round the inner side while it is still wound on the frame.
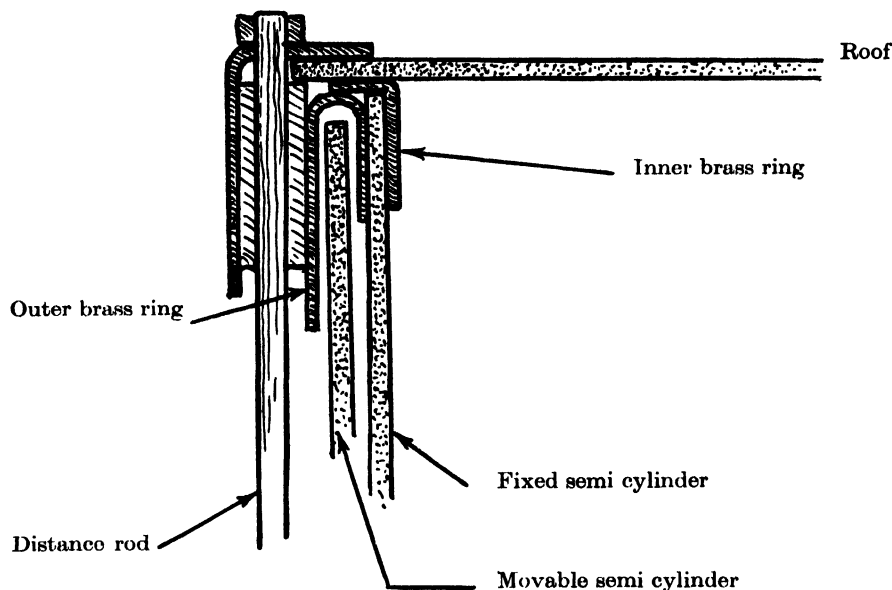

Fig. 3. –Radial section through ' hot box ' to show the arrangement of metal rings and sheet plastic.

To do this, it is necessary to pay off some inches of film at one end. The dried film still needs further cleaning. It is passed over two rotating mops of soft cotton material, the first of which is moistened with alcohol.

These films are printed by one of the firms in London which undertake this work. In such negatives there is bound to be some variation in density, partly due to the high contrast of the emulsion. I have found it necessary to make special arrangements with the film printers to grade the negative and to print it to an even density in the positive.

SUMMARY.

1. The photomicrography of living cells by phase contrast on ciné film is discussed.

2. A time-lapse apparatus for 16-mm. ciné-photomicrography is described.

3. Allusion is made to points in the practical working of such apparatus.

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DESCRIPTION OF PLATES.

PLATE I.

Figs. 1–3.—Part of a binucleate cell in a tissue culture of a mouse spleen, photographed by phase-contrast in three different ways :

    1. Enlarged to ×1400 from a negative on 16-mm. R55 film ; magnification on negative ×200.

    2. Enlarged to ×1400 from a negative on 16-mm. film for ciné-photomicrography. Magnification on negative ×200.

    3. Enlarged to ×2200 from a negative on 35-mm. film No. 1372. Magnification on negative ×700.

Fig. 4.—Phase-contrast photomicrograph of the surface of part of a mouth epithelial cell. ×4000, to show the system of surface markings.

PLATE II.

Fig. 1.—Camera unit, swung away from microscope, seen from the side. The frame counter, camera switch (B, text-fig. 2), and the actuating arm for the swinging focusing prism can be seen. (Compare with text-fig. 1.)

Fig. 2.—Camera unit ; rear view, showing the motor, and the driving chain and sprockets.

Fig. 3.—Camera unit in position over microscope, with the inclined visual eyepiece in forward position, out of use. On the left is the thermo-regulator.

Fig. 4.—Illuminating unit. The electromagnetic shutter is in position in front of the field iris.

Fig. 5.—The " hot-box " open. Within is the microscope, mounted on the heating table. Some of the extensions to the microscope controls are seen on the left.

Fig. 6.—Rear view of apparatus, showing the rotating disc of neutral filters, and the supports for the vertical column, which carries the camera head.

Fig. 7.—View of a master controller, in which a mercury switch carried on an arm is operated by a set of rotating cams. One with three gaps is seen.

VIII.—THE CULTIVATION *IN VITRO* OF VARIOUS AMPHIBIAN TISSUES.

By M. M'E. Preston.

(Strangeways Research Laboratory, Cambridge ; Wheatstone Physics Laboratory, King's College, London.)

Two Plates.

Introduction.

The cells of tissue cultures are very favourable for study by modern optical methods such as phase-contrast and ultra-violet microscopy, as they are thin, flat, and very transparent ; they divide readily and are suitable for the study of mitosis and chromosome structure. Amphibian cells are considerably larger than those of birds and mammals and have relatively few chromosomes (Wickbom, 1945), and it seemed probable that cultures of amphibian tissues would prove especially valuable for certain types of bio-physical investigation. The following experiments were made to find suitable methods of cultivating amphibian cells and to compare the behaviour *in vitro* of tissues from different organs and different species.

Amphibian tissue has been cultivated by several workers. The earliest cultures of amphibian material were made by Ross Harrison (1907, 1910), when he showed that the axon of the nerve cell developed as a protrusion of the protoplasm of the cell. In these experiments he used explants of neural tube from 3–6 mm. embryos of *Rana sylvatica* and *Rana palustris*, and the cultures were made in a medium of coagulated lymph. Drew (1913) explanted the kidney and spleen of the adult frog (*Rana temporaria*) ; leucocytes and endothelium migrated from the kidney and ciliated epithelium was formed from the nephrostomes ; leucocytes and fibroblasts emerged from the spleen. Holmes (1913, 1914), using a medium composed of blood serum and Grübler's gelatin, obtained epithelial outgrowth with mitosis from the skin of adult frogs and from larval ectoderm (*Rana, Triton, Oymetylus, Salamandra*). Uhlenhuth (1914, 1915) studied the effect of the consistency of the medium on the character of the outgrowth in cultures of the regenerating tail of tadpoles (*Rana pipiens*). Wermel (1931) describes the outgrowth of fibroblasts, wandering cells, and some spermatogenic material from explants of the testis from adult *Rana temporaria*. Another report of differentiated growth in culture is that of Nassanov (1934), in which he reports the formation of cartilage by connective tissues of *Axolotl*.

<center>MATERIAL AND METHODS.</center>

1. *Tissue.* Cultures of the following tissues were made :

Rana temporaria : spleen, liver, kidney, testis, muscle, skin, brain, bone marrow.

Xenopus lœvis (tadpoles ; 3–6 weeks old) : limb, kidney, nerve cord.

Triton vulgaris, T. palmata, T. cristata : liver, kidney, limb, brain, muscle.

2. *Dissection.*

Rana and *Triton* were killed and washed thoroughly with 80 p.c. alcohol ; skin was removed from the ventral body-wall, which was then opened to expose the viscera. Explants of skin of *Rana* were made and sterilized by immersion in 20-vol. hydrogen peroxide. The method was not always successful.

The *Xenopus* larvæ were killed and dissected in Tyrode diluted to the concentration of amphibian Ringer and containing 3000 units per ml. of penicillin ; the tail was cut off and the tissues for explantation were removed with the aid of a cataract knife and a fine needle. The kidney, nerve cord, and part of the developing limbs were stored for 24 hours at 4° C. in saline containing 1000 units per ml. of penicillin, which helped to sterilize the tissues.

3. *Culture Methods.*

The explants were grown in hanging drop preparations on 1¼ inch square cover-slips sealed with molten paraffin wax over 3 × 1½ inch hollow-ground slides.

In preliminary experiments it was found that in cultures of frog tissue, cell migration and mitosis would take place in a medium in which the salt concentration was higher than that of amphibian physiological saline (0·65 p.c. NaCl). For most of the investigations, however, appropriate adjustments in the salt concentrations of the medium were made. Tyrode diluted with glass distilled water to give a concentration of 0·6 p.c. NaCl was used for all except preliminary experiments. The following culture media were prepared.

Medium 1.—Chicken plasma was diluted with glass-distilled water (6 parts plasma, 2 parts water) to give approximately the salt concentration of amphibian blood. Saline extracts of an 11-day chick embryo and of frog-leg muscle were made by the standard method and were then mixed (10 parts frog muscle extract, 1 part chick embryo extract). For the final culture medium one drop of this mixture was added to one drop of plasma. The presence of the small trace of chick embryo extract was found to reduce the clotting time from about 20 minutes to 30 seconds.

Medium 2.—The salt concentration of the plasma was adjusted with distilled water as in medium 1 to produce a softer clot in the final medium that would be more readily penetrated by the migrating cells, the plasma was diluted with saline in the proportion of 1 part saline : 2 parts plasma. Extract of a 10–12-day chick embryo was made with ordinary Tyrode's solution and was afterwards diluted to the amphibian salt concentration with distilled water. The final

medium consisted of one drop of plasma mixed with one drop of embryo extract (and for certain experiments the medium contained 100 units per ml. of penicillin).

Penicillin was always added to the culture medium when tissues of *Xenopus* larvæ were explanted, as this material was not completely sterilized by the preliminary treatment with penicillin. At the first subculture the tissue was again washed with saline containing penicillin, but it was usually unnecessary to add penicillin to the medium during subsequent cultivation.

Cultures from *Xenopus* and *Triton* grew best when incubated at 26° C., but satisfactory though slower growth was obtained at room temperature. The cultures of *Rana* tissues were grown at 20°–24° C. laboratory temperature. *Rana* and *Xenopus* tissue cultures were transferred to fresh medium every 3–4 days and those from *Triton* at 6–7 day intervals. *Rana* kidney and testis cultures were grown for 25 days, *Triton* liver cultures for 22 days, and *Xenopus* kidney and nerve for 17 days. In the case of *Rana* and *Triton* adult tissues the mitotic index fell after 17 days, but *Xenopus* cultures still showed active growth and mitosis.

RESULTS.

Rana.

Testis.—The testicular explants, which were grown in medium 1, showed a seasonal variation in the histological character of their outgrowth. Cultures prepared in June and kept at room temperature grew very little until after they had been transferred to fresh medium on the third or fourth day ; there was then a profuse outgrowth of fibroblasts and wandering cells. Migration was fairly active from the fifth day for several days, but dividing cells were never so plentiful as in cultures of avian and mammalian tissues. By keeping preparations in a vertical position the amount of fat produced by the cells was greatly reduced (cf. Jacoby, 1936) and such cultures provided excellent material for cinematographic studies of mitosis by means of phase-contrast illumination.

Cultures of the testis made in August presented a very different appearance. According to Champy (1913), there is a sharp rise in spermatogenic activity at this time of year, and among the cells which migrated from the August explants many were in different stages of sperm-formation. Sometimes vesicles appeared in the outgrowth containing up to 24 spermatids, some of which were seen to develop actively lashing tails.

Kidney.—Soon after explantation, wandering cells migrated actively from the tissue and after subcultivation fibroblasts and sometimes epithelium also appeared. Epithelium when present usually predominated over other cell types ; either it formed large sheets or smaller islets of closely packed poly-hedral cells or a growth in which the cells were not so closely associated and which somewhat resembled that of fibroblasts. Mitosis was occasionally observed in the epithelium.

Liver and bone marrow.—A few explants were made of liver and bone marrow, from which great numbers of amœboid wandering cells emerged.

Skin.—When sterile cultures were obtained the explant was surrounded by a slowly growing sheet of epithelium.

Triton.

Liver.—The explants were grown in medium 2. During the first 3–6 days the outgrowth consisted of various types of wandering cells, including macrophages with typical undulating membranes and polymorph leucocytes, some of which degenerated. Multinucleate giant cells and pigment cells were present throughout the culture period.

After subcultivation on the third to the sixth day, fibroblasts appeared in company with a variable number of macrophages and leucocytes. Fibroblasts in 4-6 day cultures although they contained much fatty material were suitable for cinematography and detailed cytological examination. Those of *T. palmata* tended to produce long, fine processes, often filled with fat globules and extending far into the medium ; this characteristic was not seen in the fibroblasts of *T. cristata.*

Xenopus Tadpole.

Limbs.—Cultures were made of the fore- and hind-limbs of *Xenopus* tadpoles aged 3–6 weeks. Explants of the early *fore-limb* before it had broken through the ectoderm gave a uniform growth of spindle-shaped fibroblasts or of epithelial sheets. During the first 3 days the fibroblasts formed a network of cells in the substance of the clot, but after subculture most of the growth was on the cover-slip.

Fragments of the *hind-limb,* which in the same animal is always further developed than the fore-leg, produced an outgrowth of fibroblasts and macrophages during the first 24 hours, and by the end of the second day had formed a sheet of cells on the cover-slips in which mitosis was seen during the third and fourth day. In some cultures the outgrowth was epithelial instead of fibroblastic ; it usually took the form of sheets of small, closely packed polyhedral cells in which mitosis was seldom observed, but which expanded so rapidly that in 48 hours the culture might attain a diameter of some 3 mm. Sometimes epithelial chords, three or four cells in width, extended radially into the medium. Occasionally nervous tissue was included in explants of the hind limb and neuroblasts and ganglion cells appeared in the zone of growth. Schwann cells similar to those described by Murray and Stout (1942) migrated from explants of the older limbs, together with small finely branched cells the identity of which has not yet been established. After subcultivation growth was rather more rapid and mitosis more plentiful ; the peak of mitotic activity, though not determined statistically, appeared to come at the end of the second day.

The fibroblasts of the limb cultures when expanded on the cover-glass were very favourable for study with phase contrast illumination. The cells were roughly triangular ; the narrow end, which pointed towards the explant, was filled with fat globules, but in the expanded distal region the mitochondria were beautifully clear. Some mitochondria appeared as black filaments, twisting and coiling as they moved up and down the cell, they frequently changed into rounded bodies which might again revert to the elongated form. The cytoplasm contained other irregular, refractive structures possibly representing Golgi material. The outline of the nucleus was not very distinct, but the nucleoli were clearly seen and were continually changing shape.

Kidney.—Explants of the kidney from *Xenopus* tadpoles of 3–6 weeks were made in medium 2. During the first 3 days many macrophages and other wandering cells emigrated from the tissue fragment, together with branched pigment cells from the surface of the organ. After subcultivation fibroblasts also appeared, and became even more abundant during the second passage ; they resembled those in the limb cultures, but contained less lipoid material and tended to spread out more on the cover-slip. Mitotic figures were most numerous on the third day after the transfer of the explant.

Epithelial sheets were formed which differed from those of the limb cultures in that the cells which were sometimes spindle-shaped and arranged parallel with each other, were less closely packed. Cords of small spindle-shaped cells, apparently under tension, and occasionally islands of small polyhedral cells isolated from the main zone of outgrowth, were also produced. Mitosis was not seen in the epithelium.

During the first and second passages ciliated cavities developed in the explants, in which particles actively circulated. Occasionally a flattened tubule composed of a single layer of ciliated epithelium, extended into the zone of outgrowth in contact with the glass surface.

Nerve cord.—Fragments of the nerve cord with its enveloping pigmented sheath were explanted. Four days later flattened fibroblast-like cells with many branched processes emerged from the cut ends of the cord.

In one culture, 5 days after the first transplantation spindle-shaped cells associated with fibres grew into a liquid area adjacent to the explant. The cells, some of which were in contact with the cover-slip, formed a cord stretching from the explant across the area of liquefaction to the coagulum on the further side, when it expanded tangentially along the curved border of the clot. Some of the cells partly retracted their thicker fibrous extensions and divided by mitosis. The coarse network of fibres which accompanied the cells at varying focal planes was under tension ; the junctions of the fibres were tri-radiate, the angles between the diverging fibres being approximately equal. Near the spindle-shaped cells described above the fibres were relatively thick ; the more distant threads were so fine as to be only just visible in the phase-contrast microscope and were attached to small, rounded, highly refractile cells.

CONCLUSION.

Amphibian tissues grew better in the modified culture media described above than when explanted into normal fowl plasma and chick embryo extract ; the cells divided more actively, accumulated less fatty material, and flattened better on the glass, which made them more suitable for cytological study.

A certain degree of differentiation was sometimes observed in the outgrowth. In some cultures this appeared to be due to the migration and multiplication of cells which were already differentiated at the time of explantation, but in others further development seemed to take place *in vitro.* Thus in the outgrowth from explants of frog testis taken in August, cysts appeared in the zone of growth containing spermatids which continued, though they did not complete their development during cultivation. This has also been described by Wermel.

Kidney explants from the adult frog and from *Xenopus* larvæ often produced sheets of polyhedral epithelial cells. Nishibe reports little or no epithelium from the kidney of adult *Bufo*. In the *Xenopus* cultures, though not in those from *Rana*, the epithelium was often organized into ciliated cysts and tubules in the neighbourhood of the explant. It would seem that the larval kidney tubules of *Xenopus* are able to continue their development *in vitro*, whereas the adult kidney, having less developmental activity, does not produce differentiated structures in the outgrowth. The tissue of larval *Xenopus* grow more rapidly and extensively than those of adult *Rana* and *Triton*.

SUMMARY.

Tissues from three species of amphibia were grown *in vitro*. A description is given of cells in the outgrowth as seen in stained preparations, and by phase contrast technique in the living state.

The growth of larval tissues of *Xenopus lœvis* is very active. Four types of epithelial outgrowth have been observed in these cultures.

Testicular explants of *Rana temporaria* gave a different type of growth according to the time of year at which the explants were made. During August, at the time of greatest spermatogenic activity, vesicles containing developing spermatids were present in the outgrowth.

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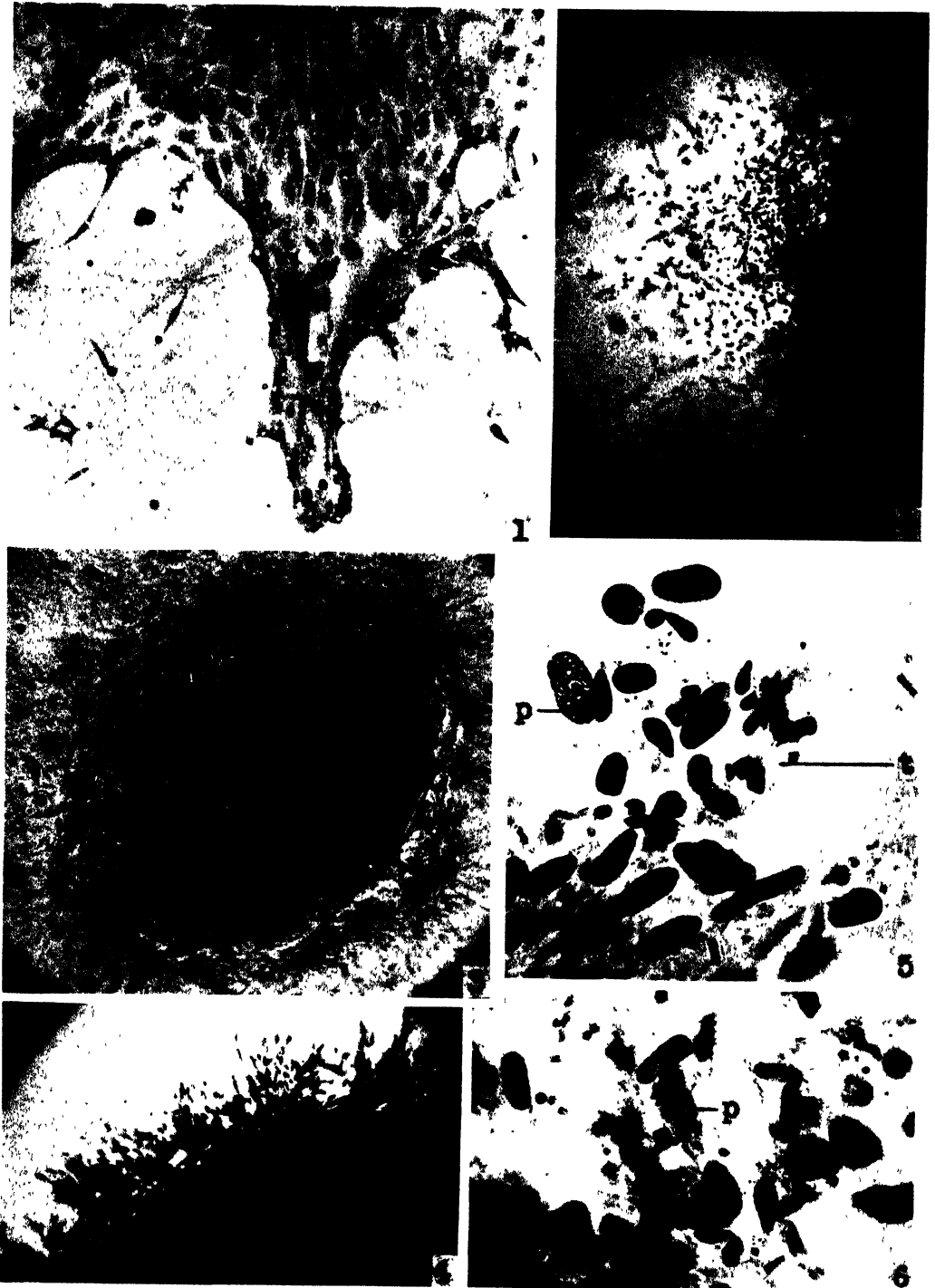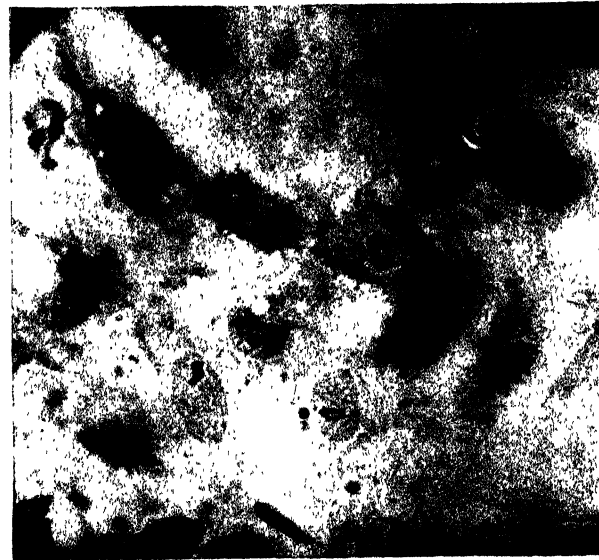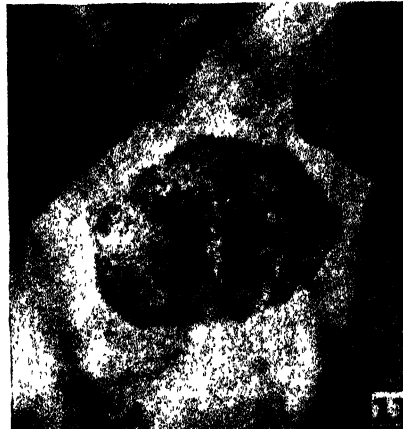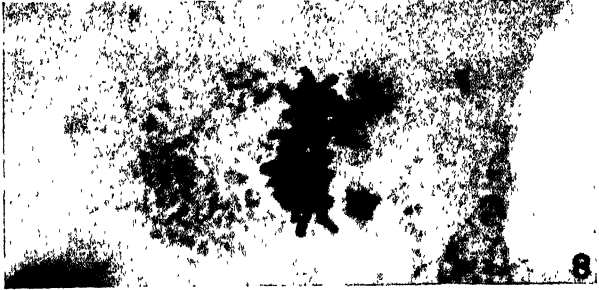
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DESCRIPTION OF PLATES.

PLATE I.

Fig. 1.—Culture of kidney from *Xenopus lævis* tadpole. Outgrowth of epithelium. (× 120.)

Fig. 2.—Culture of kidney of adult frog, first passage. Outgrowth of mesothelium and
leucocytes. (× 58.)

Fig. 3.—Culture of testis of adult frog, second passage. Showing organization within explant.
(× 58.)

Fig. 4.—Culture of testis of adult frog, three days old. Original explant. Outgrowth of
fibroblasts. (× 260.)

Fig. 5.—Culture of newt liver. Outgrowth of fibroblasts, one of which is in prophase (*p*) and
the other in telophase (*t*.) (× 260.)

Fig. 6.—Culture of newt liver. Outgrowth of fibroblasts, one of which is in late prophase (*p*).
(× 260.)

PLATE II.

Fig. 7.—Culture of adult frog kidney. Late prophase in an epithelial cell. (× 1125.)

Fig. 8.—Culture of mesenchyme from *Xenopus lævis* tadpole. Cell in metaphase. (× 1125.)

Fig. 9.—Culture of testis of adult frog. Fibroblast in anaphase. (× 720.)

Fig. 10.—Anaphase in *Xenopus lævis* culture. Two chromosomes have lagged behind.
(× 1125.)

Fig. 11.—Culture of kidney of *Xenopus lævis* tadpole. Fibroblast in anaphase. (× 1125.)

Fig. 12.—Late telophase in *Xenopus lævis* culture. (× 450.)

535.826.7   IX.—THE SUDAN BLACK B TECHNIQUE IN CYTOLOGY.

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Introduction.

In the past few months we have been using the Sudan Black B as a cytological stain. The use of Sudan dyes in cytology dates back to the time of Ciaccio (1910), who used his bichromate formol Sudan method for a variety of objects, including mammalian testis, in which the sphere or archoplasm was shown to be sudanophile. A review of these Sudan methods will be found in Lison's useful " Histochimie animale " (1936), where he advocates the replacement of Sudan III or IV by the more recently developed Sudan Black B.

Sudan Black was introduced by Lison and Dagnelie in 1935 as a myelin stain, and as a general fat stain by Gerard (1935); see Conn (1946). The latter says all that it has yet been possible to learn of this dye is that it is a complex poly-azo compound. According to Lison, " Le noir Soudane B permet cependant de distinguer entres les corps gras normaux des tissus et des hydro-carbures qui y sont présents à l'état pathologique (' vaselinomes ')." This dye was a product of I. G. Farbenindustrie. There can be no doubt, as Baker (1944) has shown, that Sudan Black will take its place with the silver and osmic methods as a stain for the cytoplasmic inclusions. We are not yet* clear as to what it stains *universally* in cells : this is to say that it has been claimed that Sudan Black always stains the Golgi apparatus, but the writers are not yet satisfied that this is so. That it stains the dense nebenkern rods or dictyosomes of invertebrate germ cells is certain, but so will Sudan III and IV. That it is a much more energetic stain of the cytoplasmic inclusions of germ and other cells is equally certain, for it will stain such cell components as deeply in a few minutes as will the red Sudan dyes in hours.

We have tried various advocated methods for Sudan Black. Among these, the one which John R. Baker (1944) refers to as his " formal Ca-Cd Sudan Black " method, consists of fixing in : formalin 10 c.c., calcium chloride anhydrous, 10 p.c. aqueous 10 c.c., cadmium chloride 10 p.c. aqueous 10 c.c., aq. dest. 70 c.c. This fluid is Aoyama's cadmium chloride formol with added calcium chloride, and Baker has not stated that this is so. It has been routine practice in this laboratory, since Aoyama's method was introduced into this country

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* We have recently got thus far : Sudan Black does not stain the vertebrate neurone Golgi apparatus, but it does stain a peri-nuclear granulation, which may be the basis of the vital coloration in neutral red and methylene blue. Sudan black stains the archoplasm of the rat spermatocyte a grey colour, and surface granules on this are black ; mitochondria are greyish black, von Ebner's granules quite black (March 14th, 1949).

about 1931, to fix material in formol cadmium chloride, to silver part of it, and to carry this and unsilvered pieces through to the frozen section Sudan IV method. This was done not so much because we thought that cadmium chloride in some way assisted Sudan staining, but because it cut down the number of bottles of fixatives necessary for research work on the Golgi apparatus and fats, and seemed to give a somewhat improved fixation, though there is much doubt about this, especially in the case of 3-day fixed formol material; nor is the amount of cadmium chloride adjusted correctly for the osmotic pressure of the tissues. While it might appear useful to have used formol saline, we cannot see how this could be in a smear one cell layer in thickness. In fact, we have not been able to distinguish between smears fixed in formol Cd (Aoyama), formol alone, formol NaCl 0·75 p.c., formol calcium, or formol Ca-Cd, the formol in each case being 4 p.c.

As regards the nomenclature suggested by J. R. Baker, though it might appear terminologically sound to alter the older term " formol " to " formal," we are definitely against this, and we regret to see the editors of the *Quarterly Journal of Microscopical Science* publishing papers side by side in some of which the word " formal " is used and in others the word " formol." " Formal " is a synonym for methylal or methylen dimethyl ether, $CH_2(O.CH_3)_2$, and is stated to be so in dictionaries of chemical terminology.

The Sudan Black B method has been studied by the histologist E. H. Leach (1938). His schedule is as follows: fix for 24 hours in 5 p.c. formol saline. Wash in running water for 24 hours. Cut frozen sections 5–10μ straight away or after embedding in gelatine. Transfer to aq. dest., then into 50 p.c. diacetin (glycerol acetate used by Gross as a Sudan solvent, Carleton and Leach 1939), agitate for a few seconds, transfer to Sudan Black solution, made up previously as follows: add excess I.G.F. Sudan Black to equal parts of diacetin and aq. dest. and incubate for 2 days at 55° C. Cool and filter just the right amount for staining. Thickish sections need 2 hours in stain, but staining may be hastened by warmth. Transfer to 40 p.c. diacetin for 30 seconds, pass to water; counterstain in carmalum. Float sections on to slide, mop, mount in Apathy's medium. This is a sound method, especially suitable for vertebrate material.

In our studies we have used smears of *Helix aspersa*, a *Limnœa stagnalis*, and *Lumbricus terrestris* germ cells, and what we have to say below about the various Sudan techniques is based on such material alone. Nevertheless, to all those working on protozoa and germ cells, a critique of these smear techniques is important.

We first tried to find out whether the addition of cadmium chloride and calcium chloride was an advantage in formol fixation. We already believed, since 1931, that the addition of cadmium chloride did not inhibit Sudan IV staining, because the use of Aoyama's fluid had become routine in our studies. We found immediately, in these smears, that Sudan Black (hereafter called S.B.) stained any formol fixed material so energetically that it was not possible to say whether there was any improvement when using the times given by Baker. We turned therefore to Sudan IV (hereafter called S. IV), which is less energetic, and noted that the addition of cadmium chloride slightly inhibited staining with S. IV and that calcium chloride did not make any difference. In these cases

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the fixation period was varied from 3 hours to 3 days. Our best S. IV preparations of *Helix*, using plain 4 p.c. formol, were from material fixed 3 days and stained overnight in S. IV. It should be noted that this result refers to *Helix* and not to vertebrate material.

As to the "formal Ca-Cd S.B. method" we found at once that a difficulty was the appearance of precipitate of S.B. in the finished slide. It is tiresome trying to control the process so as to keep in enough S.B. and yet to avoid a film of S.B. crystals throughout the preparation.

Moreover, formaldehyde is not the best fixative for cells exposed in a smear. The best fixative is Flemming without acetic acid, and we have used this as the basis. We were pleased to note that no precipitate appeared in our slides, and that staining could be controlled under the microscope.

Our schedule for the methods is as follows : Make thin smears on coverslips. Drop on to F.W.A. in a petri dish for 1 *minute only*. Transfer to running water, smear side up, for 30 minutes up to 1 hour. Transfer to 50 p.c. alcohol and then to 70 p.c. alcohol for a minute or so. Filter one drop of saturated S.B. solution in 70 p.c. alcohol on to a slide. Drain a cover-slip smear quickly and remove alcohol from back of cover by pushing it across a piece of flat foolscap paper, then place the cover-slip, smear side down, on the drop of S.B. on the slide. The reason for removing the 70 p.c. alcohol from the back of the cover is to permit the use of an oil or water immersion, if a good high dry lens is not available. Now watch the staining under the microscope. Two–five minutes suffice, and no precipitates appear. With the nebenkern of *Lumbricus* cells, staining in S.B. takes place before the slide can be got under the lens. When it has been found that the staining is complete with S.B., lift the cover off with needles, dip into 50 p.c. alcohol for a few moments, and transfer to distilled water. If it is desired to tinge the nuclei, place in Mayer's carmalum for ½ minute. Mount in Apathy's syrup or Farrants' medium, then leave on warm plate for some hours.

For thick smears, the wash after the F.W.A. fixation must be prolonged for several hours—for example, this must be done for teased ganglion preparations. While it is possible to stain cover-slip and slide smears by leaving them in S.B. solution in petri dishes, we have found that the method given above is best, as it is possible to watch until the right depth of staining is reached. Leaving cover-slip smears in small quantities of S.B. solution exposed to the air and to evaporation has not given us reliable results, and is definitely not advocated.

F.W.A. and S. IV may be used in the same way. The stain takes hours to act, and the cover-slips should be left in a closed petri dish until the granules in the cells are sufficiently stained. Passage from the S. IV solution to 50 p.c. and water must be rapid, or else the dye is extracted. We should mention that Zenker without acetic acid can be used with smears, but is inferior to F.W.A. We had no success with chromic acid formol fixatives.

DISCUSSION.

The sudanophily of the Golgi apparatus dictyosomes in molluscan cells was demonstrated by Mme. Lydia Karpova in 1925 with the Ciaccio method,

and by the late William Boyle in 1937 using the formol cadmium chloride (Aoyama fluid) and S. IV method. John R. Baker (1944) confirmed this, using formol cadmium and calcium chloride fixation and S.B. In his critique of previous studies, Baker states that " Boyle (1937) showed that the chromophobe part of the Golgi element in the neurones of *Helix* can be coloured by Sudan IV in frozen sections of material treated by Aoyama's technique." This is misleading, as Boyle (p. 246) states that with formol-Sudan " there appears a large number of straight or curved rod-shaped bodies stained bright red (pl. 1, fig. 8). These agree closely in number and position with the distribution of the Golgi elements." Boyle does, however, in addition point out that in Aoyama silvered preparations the dictyosome is blackened and the chromophobe area goes red in Sudan. We have found that with formol-fixed invertebrate neurones the edge of the dictyosome is more sudanophil than the chromophobe area, which parts with its Sudan quite rapidly on diffentiating in 50 p.c. alcohol and then appears as an unstained vacuole. We doubt whether it is a vacuole. According to Baker, S.B. shows masked fats as well as fat droplets. We are doubtful as to whether this is correct. In our experience S.B. stains the same things as S. IV, but the latter takes much longer to act and may need heat. There can be no doubt that the S.B. method will be very useful. It is interesting to note that if the F.W.A.-fixed smears are taken out of 70 p.c. alcohol and stained by Heidenhain's method (½ hour alum, 1 hour haematoxylin), an adequate stain of the cytoplasmic inclusions of *Helix* cells is obtained. For vertebrate material there is no technique which for usefulness at present equals the combined S. IV and Aoyama silver nitrate method, because with it the Golgi material is golden brown to black and glycerides are red.

SUMMARY.

A schedule is given for staining smears with Sudan Black after Flemming without acetic acid fixation. This method is easily controlled and has the merit of a proper fixation of the smear.

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582.622.2 X.—PEPPER AND WHITE PEPPER SHELL.*

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Two TEXT-FIGURES.

WHITE pepper consists of the fruits of *Piper nigrum* Linn. from which the outer part of the pericarp, as far as the position of the vascular strands, has been removed. The bulk of this commodity is sold as powdered white pepper, which occurs commercially in different grades. The finest grade consists almost entirely of the finely powdered perisperm of the peppercorn. To obtain this high-grade product, white peppercorns are decorticated to remove the remnant of the pericarp and also the few layers of the very thin seed-coat. The layers removed form collectively " white pepper shell," which when powdered has been used as an adulterant of powdered white pepper. This adulterant, being a part of the white peppercorn, has proved to be very difficult of detection and the determination of the amount added has presented an almost insoluble problem.

The proportions of non-volatile ether extract, of starch, and of crude fibre have been used as criteria for powdered white pepper (Federal Standards, U.S. Dept. Agric., quoted by Woodman 1941). These values, however, cannot be successfully used to determine the proportion of added white pepper shell, because an addition of as much as 20 p.c. of shell does not bring the contents of starch and of non-volatile ether extract below the minimum amounts obtainable from average samples of genuine white pepper. The crude fibre of commercial samples of white pepper shell does not appear to have been recorded, the husk or shell mentioned in the literature of crude fibre values seems to have referred to the husk of black pepper.

NUMBER OF BEAKER CELLS PER MG. OF WHITE PEPPER SHELL.

The layer of sclerenchymatous beaker cells (see fig. 1, B) provides a means of characterizing white pepper shell, and experiments were made to discover whether these cells could be used for quantitative determinations as well as for qualitative recognition of the shell. A sample of genuine white pepper shell was powdered in the laboratory to No. 85 fineness and was used for experiments to determine the number of beaker cells per mg. of white pepper shell. Three

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* The subject matter of this communication forms part of a thesis accepted for the degree of Ph.D. by the University of London.

suspensions were made by the lycopodium method (Wallis, 1920) as detailed for the count of sclereids in clove stalk (Wallis and Santra, 1947) and slides prepared from these were examined. The number of beaker cells per mg. of
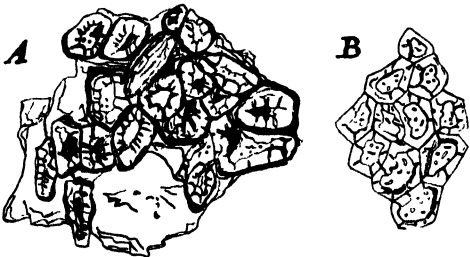

Fig. 1.—*Piper nigrum* Linn., fruit. A, hypodermal sclerenchyma of black pepper shell
    B, beaker-cell layer of white pepper shell. Both × 200.

white pepper shell was calculated from the numbers of sclereids and of lycopodium spores counted on each slide. The following is an example of the calculation, using the counts from one slide :

Weight of lycopodium   ..   ..   0·05 gm.

Weight of white pepper shell   ..   ..   ..   0·10 gm.

No. of lycopodium spores in 25 fields (=2·403 sq. mm.)   228

No. of beaker cells in 7 strips across cover-glass (=26·04 sq. mm.)   664

∴ No. of lycopodium spores in 26·04 sq. mm. .. $\dfrac{228 \times 26 \cdot 04}{2 \cdot 403} = 2471$ spores.

Since 2471 spores correspond to 664 beaker cells,
   ∴ 94,000 spores (i.e. 1 mg. of lycopodium) correspond to   ..   .. $\dfrac{94,000 \times 664}{2471}$ beaker cells.

Since 1 mg. of lycopodium corresponds to 2 mg. of white pepper shell in the above mixture,
   ∴ 1 mg. of white pepper shell contains   .. $\dfrac{94,000 \times 664}{2471 \times 2} = 12,630$ beaker cells.

The details of the counts on all slides and of the results derived from them in this way are as follows :

	No. of spores in 2·403 sq. mm.	No. of beaker cells in 26·04 sq. mm.	No. of beaker cells per mg. of white pepper shell.
Suspension I—			
Slide A	228	664	12.630 ⎫
Slide B	267	633	10,300 ⎬ 11,733
Slide C	228	645	12,270 ⎭
Suspension II—			
Slide A	283	1267	9705 ⎫
Slide B	299	1521	11,025 ⎭ 10,365
Suspension III—			
Slide A	400	1023	11,090 ⎫
Slide B	366	822	9774 ⎬ 10,250
Slide C	344	785	9885 ⎭

Grand Mean = 10,783

NUMBER OF BEAKER CELLS PER MG. OF WHITE PEPPER.

Experiments were next made to obtain the number of beaker cells per mg. of white pepper. This was determined by two methods, one the lycopodium method and the other a method based on geometrical considerations of the position and extent of the beaker-cell layer in the peppercorn. Three samples of pure white pepper were reduced to No. 85 powders in the laboratory and the number of beaker cells per mg. was determined, first by the lycopodium method, as used for white pepper shell (see above). The number of beaker cells per mg. of white pepper was calculated from the counts of lycopodium spores and of beaker cells on slides prepared from suspensions of each of the three samples of powdered white pepper.

The following is an example of calculation of the number of beaker cells per mg. of white pepper.

For this experiment the white pepper was partially extracted with light petroleum to remove the greater part of the oil so as to facilitate powdering ; 45·9 gm. of the white pepper yielded 43·4 gm. of residue.

Weight of lycopodium .. .. 0·05 gm.

Weight of pepper residue .. .. 0·20 gm., equivalent to $\dfrac{0 \cdot 20 \times 45 \cdot 9}{43 \cdot 4}$

$= 0 \cdot 2115$ gm. of the original white pepper.

No. of lycopodium spores in 25 fields ($= 2 \cdot 403$ sq. mm.) 320

No. of beaker cells in 7 strips ($= 26 \cdot 04$ sq. mm.) .. 239

Corresponding no. of spores in the same area (26·04 sq. mm.) .. .. $\dfrac{320 \times 26 \cdot 04}{2 \cdot 403} = 3468$

Since 3468 spores correspond to 239 beaker cells, ∴ 94,000 spores (i.e. 1 mg. of lycopodium) correspond to .. .. $\dfrac{94{,}000 \times 239}{3468} = 6478$ beaker cells.

But 1 mg. of lycopodium corresponds to .. $\dfrac{0 \cdot 2115}{0 \cdot 05} = 4 \cdot 23$ mg of white pepper.

∴ 1 mg. of white pepper contains .. .. $\dfrac{6478}{4 \cdot 23} = 1531$ beaker cells.

The details of the counts on all slides and of the results derived from them in this way are shown in table at the top of next page. These results show a variation of 10·5 and 6·1 p.c. above and below the mean respectively.

For the purpose of determining the *number of beaker-cells per mg. of white pepper, based upon geometrical considerations,* it was first necessary to determine the number of beaker-cells per sq. mm. of the beaker-cell layer. This was done as follows.

	No. of spores in 25 fields.	No. of beaker cells in 7 strips.	No. of beaker cells per mg. of white pepper.
SAMPLE I—			
Slide A	320	239	1531 ⎫
Slide B	304	258	1740 ⎬ 1520
Slide C	321	202	1290 ⎭
SAMPLE II—			
Suspension I—			
Slide A	150	101	1361 ⎫
Slide B	132	124	1801
Slide C	146	98	1357
			1506 ⎬ 1547
Suspension II—			
Slide A	210	160	1612
Slide B	178	140	1563
			1588 ⎭
SAMPLE III—			
Slide A	146	127	1870 ⎫
Slide B	120	105	1897 ⎬ 1789
Slide C	149	110	1600 ⎭

Grand mean = 1619

NUMBER OF BEAKER CELLS PER SQ. MM. OF BEAKER-CELL LAYER.

Several pieces of white pepper shell were mounted on a slide with chloral hydrate and by means of a camera lucida the pieces of beaker-cell layer were traced on graph paper. The number of cells in each piece was counted and the area of the pieces was determined by dividing the area traced on the graph paper by the square of the magnification. The results are as follows :

	No. of cells in the pieces of beaker-cell layer.	Area on graph paper in sq. mm.
1	28	2732
2	39	4200
3	11	1215
4	50	5634
5	7	915
6	13	1484

The total area of 148 beaker cells at a magnification of 400 diameters .. .. 16,180 sq. mm.

The actual area of the pieces examined .. .. $\frac{16,180}{400 \times 400} = 0.1011$ sq. mm.

Since 148 cells occur in 0.1011 sq. mm., ∴ there are .. .. $\frac{148}{0.1011} = 1464$ cells in 1 sq. mm.

Next, *the area of the beaker-cell layer in an average white peppercorn* was determined in the following way. Since the beaker-cell layer covers most of the surface of the peppercorn, the surface area of the peppercorn was first determined by measuring the diameter of the peppercorn and applying the formula for the surface area of a sphere, viz., $4\pi r^2$. In order to allow for the

error due to any discrepancy in the shape of the peppercorn, diameters along two axes, longitudinally from base to apex and transversely at the equator, were measured. These measurements were done in two ways : (*a*) by using a calibrated eyepiece micrometer, (*b*) by using a micrometer screw gauge.

(*a*) *Measurements with an eyepiece micrometer.*—Longitudinal sections were made through the centre of four previously weighed white peppercorns (from sample II) and the diameters along the two axes of the beaker-cell layer were measured. In the accompanying diagram, fig. 2 (drawn to scale), representing
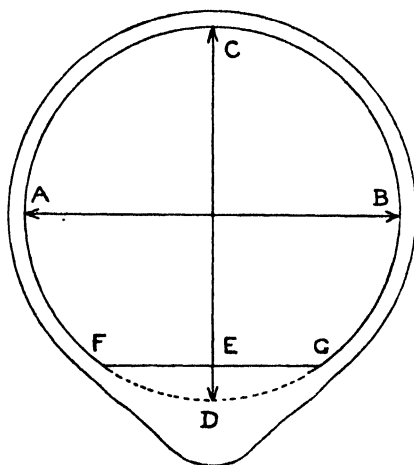
Fig. 2.—Diagram of a median longitudinal section of a peppercorn to show the extent of the beaker-cell layer, *viz.*, FACBG. For other particulars, see text. Diagram × 10.

the longitudinal section through the centre of an average white peppercorn, it is shown how the measurements were made, viz. from A to B and from C to D, representing the two diameters measured. The diameters along the two axes of each of four peppercorns expressed in eyepiece divisions (each of which is equal to $3\cdot6\mu$) are as follows :

	Longitudinal.	Transverse.	Average.	
1	1330	1420	1375	
2	1300	1100	1200	1284
3	1270	1250	1260	
4	1400	1200	1300	

The mean diameter obtained from the above experiments is therefore $1284\times3\cdot6=4622\mu=4\cdot622$ mm. Hence the surface area

$$=4\pi\left(\frac{4\cdot622}{2}\right)^2=67\cdot12 \text{ sq. mm.}$$

This area is subject to a correction because there is a circular patch at the base of the peppercorn which is devoid of beaker cells. The area of this segment of a sphere, having the radius of a white peppercorn, *viz.* 2·3 mm., is given by the formula $h2\pi r$, where h is the height of the segment and r the radius of the sphere. The height of the segment was measured by making the diagram shown in fig. 2.

which is drawn to scale at a magnification of 10 diameters. In this diagram the circular line represents the beaker-cell layer and the dotted portion from F to G is that part from which beaker cells are absent. The height of the corresponding segment is ED, which measures 4·25 mm., whence the actual height is 0·425 mm. The area of the region devoid of beaker cells is therefore $0·425 \times 2 \times \pi \times 2·31 = 6·17$ sq. mm. Hence the area covered by the beaker-cell layer is $67·12 - 6·17 = 60·95$ sq. mm. Since the four peppercorns weighed 232 mg., the average weight of one peppercorn is 58 mg., and therefore 1·0 mg. of white pepper contains $60·95 \div 58 = 1·051$ sq. mm. of beaker cells, and since 1·0 sq. mm. of beaker cells contains 1464 cells, therefore 1·0 mg. of white pepper contains $1464 \times 1·051 = 1538$ beaker cells.

This result was further verified by measuring the diameter of 100 white peppercorns from sample II with a micrometer screw gauge.

When using the gauge, the projecting part at the base of each peppercorn was removed by scraping. The average diameter of the 100 peppercorns was found to be 4·348 mm. ; the average surface area of one peppercorn is therefore 59·45 sq. mm. The area at the base devoid of beaker cells, as determined above for four peppercorns, forms $6·17 \div 67·15 = 0·0919$ of the total area. Using this figure, the area devoid of beaker cells in an average peppercorn of the 100 examined is $59·45 \times 0·0919 = 5·46$ sq. mm. This is subtracted from the total surface area, namely 59·45 sq. mm., giving the area of the beaker-cell layer as 58·99 sq. mm. in an average white peppercorn.

Since the average weight of a single white peppercorn obtained from the total weight of the 100 used is 52 mg., therefore 1·0 mg. of white pepper contains $53·99 \div 52 = 1·038$ sq. mm. of beaker-cell layer, which is equivalent to $1·038 \times 1464 = 1518$ beaker cells. The average of these two figures, namely 1538 and 1518, obtained by geometrical calculations, is 1528, which agrees closely with the figure 1547 obtained for the number of beaker cells per mg. for the same sample (sample II) of white pepper by the lycopodium method.

PROPORTION OF WHITE PEPPER SHELL IN WHITE PEPPERCORN.

Having determined the number of beaker cells per mg. of an average white peppercorn and of its shell, the normal proportion of shell in the white peppercorn was determined in the following way.

Since 1·0 mg. of white pepper of sample II contains an average of 1547 beaker cells, therefore an average white peppercorn from this sample, weighing 52 mg., contains $1547 \times 52 = 80,444$ beaker cells.

Since the beaker cells occur in the shell only of the white peppercorn and since 1·0 mg. of white pepper shell contains 10,783 cells, the weight of shell containing 80,444 beaker cells is $80,444 \div 10,783 = 7·46$ mg., which is equivalent to 14·3 p.c. of the white peppercorn.

Since the number of beaker cells in 1·0 mg. of white pepper is subject to an error of ± 3 p.c. and the number in white pepper shell to an error of ± 10 p.c., the value 14·3 is subject to an error of ± 13 p.c. and therefore represents a range of 12·44 to 16·16, or in round figures 12·5 to 16·2 p.c. *Confirmation of the result by crude fibre values.* It was first necessary to determine the crude fibre value

of white pepper shell, because this value is not available in the literature. The sample of genuine white pepper shell examined yielded 25·6 p.c. of crude fibre, as determined by the method of the Ministry of Agriculture and Fisheries. The crude fibre content of white pepper as given in the literature ranges from 3·5 to 4·7 p.c. with an average of 4·25 p.c. (Winton, 1939). Hence the average proportion of shell in the white peppercorn is 4·25 × 100 ÷ 25·6 = 16·6 p.c. with a range of 13·6 to 18·3 p.c. This result confirms the value 12·5 to 16·2 p.c. determined from the counts of beaker cells in the two commodities.

Determination of White Pepper Shell in Adulterated White Pepper.

Experiments were next made to test the possibility of applying the criteria thus obtained to determinations of white pepper shell added to white pepper. Mixtures were prepared by a fellow research worker and given to one of us (D. K. S.) for analysis. Using the lycopodium method, counts were made on slides prepared from these mixtures as described for counts of beaker cells in white pepper. The number of slides examined was adjusted to provide 35 to 40 of the larger fragments of the beaker-cell layer for each determination (Fairbairn, 1943).

The number of beaker cells per mg. of each mixture was then calculated in the following way :

Weight of lycopodium　..　..　..　..	0·05 g.
Weight of mixture 1　..　..　..　..	0·315 g.
No. of lycopodium spores in 25 fields (=2·403 sq. mm.)　..　..　..　..　..　..	294
No. of beaker cells in 28 strips from 4 slides (=104·16 sq. mm.)　..　..　..　..	2198
Corresponding No. of spores in 104·16 sq. mm. ..	294 × 104·16 ÷ 2·403 = 12,750 spores.
Hence 1·0 mg. of lycopodium (94,000 spores) corresponds to　..　..　..　..　..	94,000 × 2198 ÷ 12,750 = 16,200 beaker cells.
1·0 mg. of lycopodium corresponds to　..　..	0·315 ÷ 0·05 = 6·3 mg. of mixture I.
Hence 1·0 mg. of mixture I contains　..　..	16,200 ÷ 6·3 = 2570 beaker cells.

By a similar method the mixture II was found to contain 1976 beaker cells per mg.

Using the average figure of 10,783 beaker cells per mg. of pure white pepper shell, the total proportion of white pepper shell was calculated as follows :

(a) in mixture I, 2570 × 100 ÷ 10,783 = 23·83 p.c.
(b) in mixture II, 1976 × 100 ÷ 10,783 = 18·33 p.c.

The proportion of white pepper shell normally present in white pepper has been shown to have a mean value of 14·3 p.c. ; then, if x and y be the proportions

of added shell in the mixtures I and II respectively, the total proportion of shell in each mixture is :

$$(a)\ \text{in mixture I,}\quad x+\frac{14\cdot3}{100}(100-x)=23\cdot83$$

$$(b)\ \text{in mixture II,}\quad y+\frac{14\cdot3}{100}(100-y)=18\cdot33$$

$$\text{whence } x=11\cdot2 \text{ p.c. and } y=4\cdot7 \text{ p.c.}$$

The actual amounts present were 8 p.c. and 3 p.c. respectively.

If, instead of the mean value of 14·3 p.c., one uses the higher figure of 16·2 p.c. for the proportion of white pepper shell normally present in white pepper, these two results become $x=9\cdot1$ p.c. and $y=2\cdot5$ p.c., which are very close to the actual amounts present. The lycopodium method also possesses an advantage over calculations based on crude fibre determinations because crude fibre values allow as much as 10 p.c. of added shell to escape determination altogether.

QUANTITATIVE DISTRIBUTION OF TISSUES IN PEPPER.

The proportion of black pepper husk in the pepper fruit has been determined as 34·7 p.c. (Wallis and Santra, 1949) and that of white pepper shell in the white peppercorn as 14·3 p.c. (see above).

The proportion of white pepper shell in the pepper fruit was next determined. For this purpose the number of beaker cells per mg. of black pepper was first determined. Counts were made using a No. 85 powder as well as its air-dry crude fibre. Suspensions were made from the black pepper and the crude fibre respectively and beaker cells were counted in slides prepared from these suspensions as was done for the count of beaker cells in white pepper. The number of beaker cells per mg. of black pepper was calculated from the counts on the slides. The following is an example of calculation :

	Using No. 85 powder.	Using the crude fibre.
Weight of lycopodium	0·05 gm.	0·05 gm.
Weight of material	0·20 gm.	0·05 gm. (equivalent to $\dfrac{0\cdot05\times20\cdot4}{4\cdot4}$ gm. $=0\cdot2318$ gm. of black pepper)
No. of lycopodium spores in 25 fields (2·403 sq. mm.)	185	
No. of beaker cells in 7 strips across cover-glass (26·04 sq. mm.) ..	129	
Corresponding no. of spores in 26·04 sq. mm.	$\dfrac{185\times26\cdot94}{2\cdot403}=2205$	

Since 2205 spores correspond to 129 beaker cells, ∴ 94,000 spores

(1 mg. of lycopodium) correspond to .. .. $\dfrac{94,000 \times 129}{2205}$

$= 6040$ beaker cells.

Since 1 mg. of lycopodium corresponds to $\dfrac{0.20}{0.05}$ mg. of black pepper,

∴ 1 mg. of black pepper contains .. .. $\dfrac{6040}{4} = 1510$ beaker cells.

The details of the counts on the slides prepared both from the No. 85 powder of black pepper and from its crude fibre and the details of the results derived from them in this way are as follows :

<div align="center">EXPERIMENT WITH THE No. 85 POWDER.</div>

	No. of lycopodium spores in 25 fields.	No. of beaker cells	
		in 7 strips.	per mg. of black pepper.
Suspension I—			
Slide A	185	129	1510 ⎫
Slide B	205	125	1326 ⎬ 1492
Slide C	164	124	1640 ⎭
Suspension II—			
Slide A	157	120	1657 ⎫
Slide B	198	119	1303 ⎬ 1415
Slide C	221	131	1285 ⎭

<div align="center">EXPERIMENT WITH THE CRUDE FIBRE.</div>

	No. of lycopodium spores in 25 fields.	in 7 strips.	per mg. of black pepper.
Suspension I—			
Slide A	231	215	1741 ⎫
Slide B	210	167	1488 ⎬ 1558
Slide C	187	162	1620 ⎟
Slide D	208	154	1385 ⎭
Suspension II—			
Slide A	112	101	1687 ⎫
Slide B	120	85	1325 ⎬ 1538
Slide C	112	96	1604 ⎭

<div align="right">The mean of all average determinations   1500</div>

Since pure white pepper shell contains an average of 10,783 beaker cells per mg., therefore the proportion of white pepper shell in the pepper fruit $\dfrac{1500 \times 100}{10,783} = 13.91$ p.c.

Next, the proportion of perisperm in the pepper fruit was determined by subtracting from one hundred the sum of the proportions of black pepper shell and of white pepper shell in black pepper, *viz.* :

<div align="center">$100 - (34.3 + 13.9) = 51.8$ p.c.</div>

Similarly the proportion of perisperm in the white peppercorn was determined by subtracting from one hundred the proportion of white pepper shell in white pepper, *viz.* :

$$100 - 14\cdot3 = 85\cdot7 \text{ p.c.}$$

SUMMARY.

1. The determination of the percentage proportions by weight of genuine pepper and of the by-products of pepper-grinding, in commercial ground peppers has been effected microscopically by the use of the lycopodium method.

2. Small quantities of pepper shells and husks cannot be entirely overlooked when this microscopical method is used, whereas methods based upon the content of crude fibre, non-volatile ether extract, or starch fail to detect appreciable amounts.

3. The proportions of the various tissues present in genuine black pepper and in white pepper have also been successfully determined.

4. The numerical results obtained are summarized as follows :

Number of beaker cells (sclereids) per mg. of :

Black pepper	1453 to 1500 to 1548
White pepper	1520 to 1619 to 1789
White pepper shell	10,783 (10 p.c. error)

Number of beaker cells per sq. mm. of beaker-cell layer :   1224 to 1464 to 1626

Crude fibre of white pepper shell : 25·6 p.c.

Quantitative distribution of tissues in pepper fruit (black pepper) :

Black pepper shell in pepper fruit	34·6 p.c.
White pepper shell in pepper fruit	13·9 p.c.
Perisperm in pepper fruit	51·5 p.c.
White pepper shell in white pepper	14·3 p.c.
Perisperm in white pepper	85·7 p.c.

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535.822.98   XI.—COMMENTS REGARDING THE McARTHUR INVERTED
MICROSCOPE.

By G. D. HANNA, F.R.M.S.

(California Academy of Sciences.)

THE recent appearance of a second article by the designer of this remarkable instrument will undoubtedly appeal to the imagination of many microscopists. Those who have been obliged to carry a standard instrument in their travels and to worry about its safety will welcome the possibility of eventually obtaining such a piece of portable equipment. The author (McArthur, 1947) has given sufficient detail so that the construction should not be particularly difficult for a shop equipped with lathe, drill, and milling machine. He has also listed the advantages and disadvantages explicitly. In order that the most useful final design or designs may be made available at as early a date as possible, it would seem that comments by prospective users may be worth recording. It is with this in mind that the following ideas are offered.

Apparently the instrument the author described and illustrated is very light compared to a standard research microscope. While admitting that there is no substitute for mass to obtain rigidity, lightness is also of prime importance for certain classes of work. Therefore, although brass is the instrument-maker's preferred metal, the greater part of the new instrument could well be made of magnesium or aluminium alloys and thus again reduce the weight by about half. This would be an advantage for travelling-bag packing.

It is not particularly difficult and would scarcely add to the cost to incline the ocular backward towards the operator about 15°. This would add to the ease and comfort of the operator, especially when long periods of observation are necessary. If the ocular be fitted to the tube with sufficient accuracy it can be raised or lowered for tube length correction. Possibly, however, it would be better to use an accessory graduated sleeve for this purpose.

The author chose two right-angle prisms to divert the light beam 180°. Some advantage would be gained by coating the entrant and exit faces of these prisms with magnesium fluoride or cryolite. Also, it would help somewhat to coat the hypotenuse faces with aluminium and paint all ground surfaces dull black.

When a converging beam of light, such as that from an objective, must be bent at a right-angle there are good optical reasons for choosing some other type of reflector than the right-angle prism. The most common method is to use a penta prism or an optically flat, first-surface mirror, the latter often ground to an elliptical shape. Mirrors have the decided advantage that the

type and homogeneity of the glass is not of first importance. Prisms of any type used in a magnifying instrument should be made of excellent material and the workmanship on the flat surfaces of any such reflectors should be of excellent quality. Departure from a plane can well be held to not more than one-twentieth of a wavelength of yellow or green light.

In order to get complete inversion of the image in such an instrument it is necessary to depart from the use of two simple reflectors such as the three types mentioned. In one method a right-angle prism would be placed at one corner and a " roof " prism (sometimes called a triple reflector) at the other. Another method calls for grinding a right angle " roof " on one of the reflecting faces of a penta-prism for one corner and using an ordinary penta or flat mirror at the other. Other methods call for various forms of porro prisms such as are used in binocular telescopes, but these would be somewhat unhandy to mount in this case. It would seem doubtful if, for ordinary microscopical work, the image needs to be erected. There may be special branches where this is desirable, but the urgency would necessarily have to be sufficient to warrant the additional cost of production. Two plain, optically flat, first-surface mirrors appeal strongly to the present writer.

REFERENCE.

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ABSTRACTS.

MICROSCOPES AND MICROSCOPY.

Ciné-photomicrography.—J. DRAGESCO ("Microcinématographie sur films étroits," *Microscopie*, 1, No. 2, 1949, 10–40). Research in ciné-photomicrography, originally a French technique, is now taking place in many countries. In spite of the advantage of the standard 35-mm. film, there are many users of the sub-standard 16-mm. film, which is less expensive. The taking of 16-mm. micro-moving pictures demands a special technique. The microscope should be of high quality and fitted with apochromatic objectives, low-power compensating eyepieces, and a long-focus achromatic condenser. The 16-mm. camera should be a steady and reliable one, operated by clockwork or an electric motor and fitted with a lens focusing on the back of the film.

Methods of focusing the microscopic image are described critically, as are also the many sources of illumination which may be used. Frequency of exposure may be modified, either mechanically or electrically, so as to obtain accelerated or slow-motion pictures. Microscope and camera are screwed on to independent bases in order to suppress vibration. Photometers and colour filters may be very useful. Before the actual film is exposed the biological material should be prepared, the different sequences noted down, the correct emulsion selected, and trial shots developed.

Several types of equipment suitable for protozoological research are described. The author concludes that the sub-standard 16-mm. film is a reliable material for research or educational ciné-micrography. A list of recent 16-mm. films is appended, together with a comprehensive bibliography. B. O. P.

Ciné-photomicrography.—J. DRAGESCO ("Eine bewegliche mikrokinematographische Einrichtung," *Mikroscopie*, 3, Nos. 7/8, 1948, 238–42). An attachment is described, by the use of which an ordinary bench microscope is adapted for ciné-photomicrography. The image-forming rays are directed from the microscope eye-piece on to a mirror which reflects 85 p.c. of the incident light into the camera, the transmitted light forming an image in a viewing eyepiece. Light intensity can be measured by means of a photo-cell. B. O. P.

New Technique.—M. LOQUIN ("Nouvelle technique d'étude des structures infra-microscopique: polarisation du rayonnement de fluorescence en lumière ordinaire," *Microscopie*, 1, No. 2, 1948, 46–7). The fluorescence emitted by certain structures in the colloidal state, illuminated by dark-field methods by ordinary white light, is polarized. This property has been used to investigate the anisotropy of the structures. B. O. P.

Phase-contrast Microscopy.—M. LOQUIN ("Plaque de phase à absorption réglable en lumière polarisée," *Microscopie*, 1, No. 2, 1948, 47–8). A phase plate

embodying an annulus of cellophane having a retardation of half a wavelength is used in conjunction with a polarizer and analyser to obtain variable amplitude phase-contrast effects. B. O. P:

Phase-contrast Microscopy.—M. Loquin ("A propos de l'objective dit 'A reflecteur interne' de Spierer," *Microscopie*, 1, No. 2, 1948, 49–50). A combination of objective and illuminator described by Spierer over twenty years ago is, in effect, a primitive and imperfect phase-contrast system. B. O. P.

Phase-contrast Microscopy.—A. DE GRAMONT ("Comment réaliser un contraste de phase," *Microscopie*, 1, No. 2, 1948, 62–4). A method of producing phase-contrast effects in polarized light is described. The phase plate consists of suitably orientated half-wave retardation elements and amplitude control is obtained by the rotation of an analyser. The relative phases of the direct and diffracted beams are controlled by means of a Bravais compensator introduced between the phase plate and the analyser. B. O. P.

Phase-contrast Microscopy.—F. W. Cuckow ("The Phase-contrast Incident Light Microscope," *J. Iron & Steel Inst.*, 161, part 1, 1949, 1–10). A review is given of experiments in the comparative microscopy of metals, leading to the conclusion that new information can be gained from a knowledge of the various levels existing in the surface of the prepared metallurgical specimen. The means already available for the assessment of these levels are discussed. These include the use of the fine-focusing adjustment of the microscope, the surface finish recorder, and the interference microscope. A new instrument for this work, a phase-contrast incident-light microscope, is then described. This has been used in conjunction with a 1·8-mm. oil-immersion objective to obtain photographs at high magnification. It is likely to be particularly useful as a pilot instrument in electron metallography, as it gives images similar to those obtained from plastic film replicas. A comparative microscope is also described in which the properties of polarized light are employed to divide the field into two parts, one of which is seen under phase-contrast conditions and the other under normal microscopical conditions. The dividing line can be moved across the field of view. Photomicrographs from the instrument are shown. These indicate that it is a useful addition to the tools available to the metallurgist. B. O. P.

Phase-contrast Microscopy.—D. A. Kempson, O. L. Thomas, and John R. Baker ("A Simple Method for Phase-contrast Microscopy," *Quart. J. micr. Sci.*, 1948, 89, 351–8). The method enables anyone to use phase-contrast microscopy without having to obtain special objectives or condensers. The most perfect objectives for studying living cells in body fluid or saline solutions are water-immersion lenses and for the purpose a Zeiss 2·5-mm. apochromatic objective of N.A. I.25 was used. The phase plate was placed in a funnel stop in the objective to lie *behind* (*above*) the back focal plane of the objective. A circle of optically plane glass 1 mm. thick and the same diameter as the back lens was obtained, the one used is 5·5 mm. in diameter, and after preparation was placed in a cell to hold it within 0·77 mm. from the back lens of the objective. A diagram illustrates this. The cell was designed not to reduce the N.A. of the objective and served to hold the phase plate while processing ; the end carrying the phase plate was turned down very thin and slit in several places to function as a spring chuck or collet; this latter allows for several plates to be used interchangeably. The surface of the phase plate is sent to an optical firm for providing a service for blooming photographic lenses, and as the thickness of the bloom is not

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always the same, it is a good plan to have several plates prepared. The bloom is removed completely except for the annulus by placing the plate in the funnel stop and mounting on to a micro-turntable in a simple chuck made of brass tubing with three set-screws ; the chuck is fastened to the turntable with sealing-wax or plasticine.

The annulus is turned off with a chisel-pointed needle held in the hand or mounted on to an L-shaped lever designed for the purpose, which latter may be fastened by means of a screw through a hole at the angle of the L to a piece of wood designed to fit the hand-rest of the turntable. The coating is very carefully removed to leave an annulus (the one used by the authors is 2·58 mm. outside and 1·52 mm. inside diameter, and 0·53 mm. wide. A dissecting binocular microscope is used to control this operation. To balance the direct light coming through the annulus with that of the diffracted beam, carbon to reduce transmission is deposited on the annulus with a small flame, such as a cigarette-lighter with benzine or xylol in the fuel. Overheating must be avoided, and the plate may be smoked gradually to the desired density. The carbon is removed from the clear areas of the plate by the previously mentioned scraping technique.

The annulus for the illuminating system is constructed from a piece of glass in the path of the light with a semi-transparent annulus thereon cut from black paper. The glass should be 8 cm. square, and from the piece of black paper a circle is cut 39 mm. in diameter this in turn is cut down to a diameter of 27·5 mm. and fastened accurately in the centre of the glass, leaving an annulus 5·8 mm. wide. The whole is covered with tracing-paper damped with paraffin. An intense source of light is necessary, and for the purpose a 6 v. bulb is used focused on to the annulus with a condenser ; an ordinary ground-glass screen is suitable for preliminary adjustments and the necessary position is found by several trial adjustments. The objective annulus is observed with a 3-in. objective screwed into the draw-tube of the microscope and the necessary adjustments of the condenser and annulus screen performed by this means. The account continues : The object is to make the two annuli coincide. This is achieved (1) by adjusting the mirror ; (2) by making the bright annulus near to or farther from the mirror along the line joining the mirror to the bulls eye ; and (3) by making corresponding movements of the condenser so as to keep the bright annulus in focus. A stainless steel mirror is recommended in place of a glass one, and with a well corrected condenser of medium power, the bright annulus may be placed 20 cm. from the mirror. The method produces negative phase contrast, nuclei appear lighter than the cytoplasm and mitochondria and bacteria are particularly bright. A resistance attached to the light allows for a useful variation in intensity.   F. C. G.

New Principle.—D. GABOR (" A New Microscopic Principle," *Nature*, 1948, **161**, 777–8). When an object illuminated by an electron beam from a point focus is placed a short distance from the latter and is followed by a photographic plate at a large multiple of this distance, the interference diagram produced in the plane of the plate can be recorded. The effects are due to interference between the primary wave-front from the focus and the secondary waves emitted by the object. The photograph is developed by reversal or printed and illuminated with an optical imitation of the electric wave. On looking through it the original object is seen as though it were in place. The diagrams produce records of three-dimensional as well as plane objects. The principle has been tested by means of an optical model and is now being applied to the electron microscope.   B. O. P.

Phase-contrast Microscopy.—F. SMITHSON (" The Application of Phase-contrast Microscopy to Mineralogy and Petrology," *Min. Mag.*, 1948, **28**, 384–91).

Certain features of rock-sections are shown up more clearly when viewed by phase-contrast methods with and without polarized light, whilst other characteristics are more easily demonstrated under the ordinary microscope. A short account is given of the petrological applications in which the method seems likely to prove most useful and examples of the effects to be obtained are shown in a series of photo-micrographs. Contrast methods not involving phase-difference are also discussed. B. O. P.

Micro-hardness Testing.—E. W. TAYLOR (" Micro-hardness Testing of Metals," *J. Inst. Metals*, 1948, **74**, 493–500). Reference is made to the methods normally employed for testing the hardness of metals and alloys and attention is directed to the fact that when an indenter is used the impressions made are usually large in relation to the micro-structure of the specimen. The desirability is stressed of a form of hardness test which can be applied to a particular crystal or to a small selected area, and the factors controlling the application of very light loads to a diamond indenter are examined. A new micro-hardness tester is described, the design of which closely follows the principle discussed earlier. A number of examples are given of micro-hardness tests variously applied to small selected areas of particular specimens. B. O. P.

HISTOLOGICAL AND CYTOLOGICAL TECHNIQUE.

Cytological Methods for *Trillium erectum*.—A. H. SPARROW, and R. C. SPARROW (" Treatment of *Trillium erectum* prior to and during mass production of permanent smear preparations." *Stain Technol*, 1949, **24**, 47–55; 11 refs., 6 figs.). Schedules are given for demonstrating meiotic divisions, microspore divisions, and pollen tube divisions by means of aceto- or propiono-carmine smears. Normal meiosis occurs during storage of the plants at 4–6° C. G. M. F.

Histological Procedure for Tissue Culture.—A. COHEN, and C. WAYMOUTH (" A New Histological Procedure for Whole Tissue Cultures Grown in Plasma," *Science*, 1948, **108**, 480–81 ; 4 refs., 2 figs.). Textbook procedures for fixing and staining whole tissue cultures in plasma coagula have been unsatisfactory. An improved procedure is as follows. The culture in its coagulum on a cover-slip is fixed overnight in 3 to 4 p.c. formol containing 0·5 p.c. acetic acid or in Carnoy's fluid for 1 hour. Acetic-alcohol fixation gives a more granular and more opaque dried specimen than acetic-formol fixations. The cover-slip is then washed thoroughly in running water after acetic-formol, or in descending strengths of alcohol and water after Carnoy. After a final rinse in distilled water the cover-slips are laid flat on a glass surface protected from dust and allowed to dry slowly and thoroughly in air. Cultures grown in a fluid medium should not be dried. The dried cultures may be stained with any one of the hæmatoxylins for from 5 to 15 minutes, depending on the strength of the stain. Weigert's iron-hæmatoxylin is satisfactory. Once stained the cultures must not be allowed to dry again. Cover-slips are passed through the alcohols, cleared in xylol, and mounted. If a Leishman or Giemsa stain is used, acetone and acetone-xylol dehydrations must be used instead of the alcohols. G. M. F.

Orientating Nematodes for Sectioning.—C. OVERGAARD (" A Simple Method for Orientating Small Objects for Sectioning with Special Regard to Nematodes," *Quart. J. micr. Sci.*, 1948, **89**, 437–8). The nematode is heat-paralysed and pipetted into Bouin's mixture. After 24 hours' fixation it is passed through alcohols, aniline,

bergamot oil, and paraffin wax. A square glass plate 2 mm. thick and with a side of 22 mm., provided with a central circular hole of 15 mm. diameter, is fixed by means of Canada balsam to a microscope slide. The hole is filled with paraffin wax II, into which the object is transferred from paraffin wax I for orientation and embedding. By means of a heated needle the object is placed 1 mm. above the bottom in the paraffin wax, which is kept in a liquid state by heating over a small flame. These manipulations are carried out under a binocular microscope. When the object has been placed in position the slide is plunged into water at 10°–12° C. When the paraffin wax has become solid a block with sides about 5 mm. long is cut out enclosing the object. A block of wood with a block of paraffin wax fused on it is placed in the microtome and sections are cut till a convenient area of its surface is plane. The object block with the plane lower surface downwards is held 5 mm. above the block in the microtome. A very hot lancet is placed in the interspace between the two blocks, but without touching. When the radiant heat has melted a fine film of paraffin wax on the two surfaces, the blocks are fused to each other. After cooling the block-holder of the microtome is lowered 2 mm. by a screw. Sectioning may then take place. If the radiant heat has been applied accurately the plane of section will be parallel to the plane lower surface of the block containing the object. G. M. F.

Ester Wax as an Embedding Medium.—J. D. SMYTH, and C. A. HOPKINS ("Ester Wax as a Medium for Embedding Tissue for the Histological Demonstration of Glycogen," *Quart. J. micr. Sci.*, 1948, **89**, 431–6, 12 refs.). Plerocercoid larvæ of the cestode *Ligula intestinalis* were fixed in hot (60° C.) picro-formol-alcohol (absolute alcohol saturated with picric acid 90 ml. and neutral formol 10 ml.) for 2 hours ; then passed through three changes of absolute alcohol for 12–24 hours, absolute alcohol and ester wax equal parts for 2 hours. The tissue is then placed in pure ester wax for 3 hours and in another change of pure ester wax overnight for 12 hours. Sections are cut at 5μ, flattened on albumen-water, and stained for glycogen. Sections show glycogen filling every available inter-cellular space in the cestode tissue. G. M. F.

Phosphatase Activity.—W. L. DOYLE ("Phosphatase Activity of *Drosophila* Salivary Glands," *Quart. J. micr. Sci.*, 1948, **89**, 415–19, 10 refs.). The presence of alkaline phosphatase in chromosomes was demonstrated by histochemical staining methods (Krugelis, E. J., 1942, *J. Cell. Comp. Physiol.*, 1946, **19**, 377 ; *Biol. Bull.*, **90**, 220 ; and Danielli, J. F., 1946, *Brit. J. exp. Biol.*, **22**, 110). Phosphatases in the cytoplasm and nuclei of *Drosophila* salivary glands are better preserved by fixation in absolute acetone than in 85 p.c. alcohol. Rat tissues were found by R. C. Stafford, and W. B. Atkinson, (*Science*, 1948, **107**, 279), to have a higher alkaline phosphatase activity after alcohol (80 p.c.) than after acetone fixation. Phosphatase activity is more resistant to incubation at neutrality than at pH 8·6, but in this material there is sufficient residual enzymatic activity to permit redetermination of alkaline, neutral, or acid phosphatase activity by staining methods after an initial quantitative determination. G. M. F.

Nile Blue.—A. J. CAIN ("A Further Note on Nile Blue," *Quart. J. micr. Sci.*, 1948, **89**, 429, 2 refs.). Nile blue sulphate cannot be used to distinguish between various members of the lipids. G. M. F.

Counting Megakaryocytes.—P. PIZZOLATO ("Sternal Marrow Megakaryocytes in Health and Disease," *Amer. J. clin. Path.*, 1948, **18**, 891–7, 32 refs.). L. BERMAN, A. R. AXELROD, and E. S. KUMKE ("Estimation of Megakaryocyte Content of

Aspirated Sternal Marrow," *Amer. J. clin. Path.*, 1948, **18**, 898–905, 35 refs.). These two communications review the methods at present in use for estimating the megakaryocyte content of aspirated bone marrow. Various procedures in use are based on examination of stained smears, hæmocytometer counts on aspirated fluid marrow, and examination of sections of bone marrow. Pizzolato recommends the use of a larger counting chamber than usual with a smaller dilution of fluid, thereby reducing the personal and mechanical factors of error while taking advantage of the facility and speed of the counting chamber technique. Berman *et al.*, however, are unable to demonstrate a correlation between the results obtained by a study of smears and those of the hæmocytometer method. Further, there is no evidence of correlation between counts from smears and sections or between the hæmocytometer method and counts based on examination of sections. For quantitative study of megakaryocytes in aspirated marrow the use is recommended of sections of aspirated marrow particles, but the values obtained must always be compared with those from suitable normal controls studied by identical methods. G. M. F.

Histopathologic Technique.—R. D. LILLIE (" Advances in Histopathologic Technic," *Amer. J. clin. Path.*, 1948, **18**, 867–73). This address gives a brief summary of histopathological technique since iodine was first used as a histochemical reagent for the study of starch grains by Caventou in 1826. Carmine was introduced in 1850. Ultraviolet micrography at selected wavelengths has yielded interesting morphologic details which depend on differences in the absorption spectra of various chemical cell components. Thymonucleic acid absorbs radiant energy rather strongly at about 270 mμ. Of broader application is ultraviolet fluorescence microscopy. By this means such substances as vitamin A, lipofuscins, lipins, amyloid, ceroid, riboflavin, and chlorophyll can be localized. G. M. F.

Fluorescence Microscopy and Virus Inclusions caused by Rabies, Vaccinia, and Borna Disease.—J. C. LEVADITI, P. LÉPINE, and J. AUGIER (" Étude au moyen de la microscopie en fluorescence, des inclusions intra-cellulaires acidophiles provoquées par les virus de la rage, de la vaccine, de la maladie de Borna," *Compt. rend. Acad. Sc.*, 1948, **227**, 1061–3, 5 refs., 2 figs.). The idea of fluorescence microscopy was suggested in 1911. When cells are treated with a fluorescent dye and are viewed in light of certain wavelengths various tissue structures give out secondary waves. Tissues are embedded in paraffin, sectioned, and passed through xylol and alcohols to water. Staining is then carried out with a 1 in 500 solution of thioflavine S for 30 minutes ; sections are afterwards placed for a few seconds in absolute alcohol, passed directly to toluene, and then mounted in Canada balsam, the layer of balsam being as thin as possible. Thioflavine S fluoresces when exposed to wavelengths of light between 2900 and 4100 Å., the maximum effect being when $\lambda = 3600$ Å. For direct vision a filter is required to hold back all rays except those given out by the fluorescing tissue, the maximum secondary transmission being when $\lambda = 5150$ Å. Under these conditions photomicrographs can be taken on orthochromatic plates. Negri bodies, Guarnieri bodies, and Joest-Degen bodies caused by rabies, vaccinia, and Borna disease when treated with thioflavine S all show up as azure blue, the same tint as that given by nucleoli and red cells. Cell protoplasm, nuclei, and collagen fibres are golden yellow. When viewed by fluorescence microscopy the inclusions are oval or round, without visible structure. The elementary bodies of vaccinia, fowl pox, and lymphogranuloma venereum, and the intranuclear inclusions of herpes are all of a golden yellow tint when treated by thioflavine S and viewed by fluorescence microscopy. Intracytoplasmic virus inclusions are probably made up of elementary bodies surrounded by material elaborated by the cell. G. M. F.

Dry Preparations of Vertebrate Material.—S. N. SEDRA ("Making Dry Preparations of Vertebrate Material," *Stain Technol.*, 1948, **23**, 205–8, 1 ref., 1 fig.). The method can be applied to embryos of cartilaginous fishes; amphibian eggs, gastrulæ, embryos, and tadpoles ; chick embryos. Already fixed material is brought to 70 p.c. alcohol. It is then transferred to 80 p.c. alcohol for 24 hours and to 90 p.c. alcohol for the same period. Three changes in 96 p.c. alcohol for 4–12 hours and three changes in absolute are made, followed by equal parts of absolute alcohol and xylol overnight. Three changes are made in pure xylol for 4–12 hours and thereafter tissues are placed in pure xylol for at least 2 days. The xylol is drained off and the material partly exposed to air to allow slow evaporation. G. M. F.

Protargol.—H. A. DAVENPORT ("Protargol: Old and New," *Stain Technol.*, 1948, **23**, 219–20). American-made protargol is now being used for histological staining. The staining of sections with old and new protargol was compared. At 37° C. and after 20–24 hours in the staining solutions, the old protargol stained more strongly than the new. When staining was done at 37° C. for 40–48 hours, all samples of protargol stained well and with such uniformity that it was impossible to distinguish between old and new protargols. G. M. F.

A Giemsa-Bodian Stain for Nerve Tissues.—M. MARKHAM ("A Buffered Giemsa-Bodian Stain for Neurological Material," *Stain Technol.*, 1948, **23**, 197–200, 1 fig., 7 refs.). For bringing out Nissl substance and nerve fibres in the same section a buffered Giemsa's solution can be used to counterstain material treated by Bodian's method. Bouin-perfused or formalin-fixed material from mammals, amphibia, reptiles, and fish was used. All specimens were allowed to stand for a week in decalcifying solutions to remove all traces of calcium. The best solution is 50 p.c. formic acid 1 part, 20 p.c. sodium citrate 1 part. This solution was removed in running water. Winthrop Protargol was used for tissue staining; all glassware was cleaned with nitric acid prior to setting up the stain; before developing sections were washed in warm tap-water for 10 minutes. A gold chloride solution, 0·5 p.c., was chilled before use. Sections were placed in buffered Giemsa solution for 24 hours. As a stock solution 0·75 gm. of powdered Giemsa is dissolved in 50 ml. glycerin overnight in the oven. Then 50 ml. absolute methyl and alcohol were added. To 3 ml. of Giemsa stock solution, 87 ml. of distilled water (*p*H 5·3) were added and the solution filtered. The more alkaline the solution, the bluer the stain; the more acid the solution, the pinker the stain. After staining, sections are rinsed in 95 p.c. alcohol, two changes of absolute alcohol, two changes of xylol and mounted in clarite. G. M. F.

Alizarin Red for Bone Formation.—R. C. W. S. HOOD and W. M. NEILL ("A Modification of Alizarin Red S Technic for demonstrating Bone Formation," *Stain Technol.*, 1948, **23**, 209–18, 4 refs., 6 figs.). The alizarin red S method is modified and shortened. Embryos should preferably be fresh; if feathered birds are used the feathers must be plucked. An incision is made in the abdomen and the viscera removed from the thorax and abdomen. The prepared specimen is placed in 95 p.c. alcohol for 3 days: it is transferred to 2 p.c. potassium hydroxide till the skeletal structure shows through the muscles, approximately 3 days. The embryo is transferred without washing to a 1 in 10,000 aqueous solution of alizarin red S and stained for 6–12 hours till the embryo is a reddish purple. The specimen is transferred to 2 p.c. potassium hydroxide for 1 day and then to the following at 70° F. for 2 days; potassium hydroxide 2 p.c., formalin 0·2 p.c., pure glycerin, equal parts of all three solutions. Next the

embryo is put in 2 p.c. potassium hydroxide 100 ml., glycerin (pure) 400 ml. at 70° F. for 1 day. Finally, clearing is carried out in pure glycerin at 70° F., till properly cleared, approximately for 1 or 2 days. G. M. F.

Staining Axis Cylinders.—T. W. Adams, R. W. Thomas, and H. A. Davenport ("Staining Sections of Peripheral Nerves for Axis Cylinders and for Myelin Sheaths," *Stain Technol.*, **23**, 191–6, 4 refs, 8 figs.). Nerves are placed under slight tension and are then fixed in the following fixative for 24 hours: formalin 10 ml., saturated aqueous picric acid solution 90 ml., 25 p.c. aqueous trichloracetic acid solution 2 ml. Wash for 30 minutes in distilled water. The nerve is cut in two with a razor blade: the two portions (A and B) are treated as follows: The portion (A) to be stained with silver is placed in 70 p.c. alcohol; dehydration is carried out in graded alcohols, xylol, and paraffin. Sections are cut at 6–10μ and stained with protargol, (0·5 p.c. solution with fast green FCF added to make 0·05 p.c. dye in the final concentration. Davenport, Porter, and Thomas, *Stain Technol.*, 1947, **22**, 41). Bodian's method (*Anat. Rec.*, 1936, **65**, 89) may be used, provided metallic copper is omitted from the staining solution.

The portion (B) to be used for osmic acid staining is transferred to distilled water and washed for 30 minutes each in two or three changes. The tissue is then placed in 0·04–0·06 p.c. osmic acid solution. Very small nerves stain with the weaker solution. Staining is continued for 24 hours and well washed in distilled or running tap-water. Tissues are dehydrated, embedded in paraffin, and sectioned. Penetration by osmic acid is limited to 1 mm. or less and the best sections may occur near the beginning of the series. The silver and osmic sections should be mirror images. G. M. F.

Acetic Acid Methods for Chromosomes.—J. G. O'Mara ("Acetic Acid Methods for Chromosome Studies at Prophase and Metaphase in Meristems," *Stain Technol.*, 1948, **23**, 201–04, 5 refs.). The root tip is removed from seed and placed in saturated and aqueous solution of monobromonaphthalene for 3 hours. The solution is poured out and replaced by a fixative with 70 parts of ethanol and 30 parts of glacial acetic acid: the material remains in the fixative for 2 or 3 days. The opaque tip is removed from the root and macerated on a slide in a drop of acetic-orcein with the edge of another slide. The material is covered, heated to near boiling, and flattened by pressing slide cover down on a thick blotter. This technique was satisfactory for *Zea*, *Impatiens*, *Crepis*, *Lotus*, and *Avena*. For detailed chromosome studies at mitosis the following schedule is recommended : the root tip is removed from germinating seed and placed in a 3 p.c. aqueous solution of methanol for 3 hours ; the solution is removed from vial and replaced with a fixative of 65 parts methanol, 5 parts chloroform, and 30 parts glacial acetic acid. Leave in fixative for 2 or 3 days. Remove the opaque tip from the root and macerate in a drop of acetic lacmoid on a slide. Cover, heat to near boiling, and flatten as before. G. M. F.

Phosphotungstic Acid Hæmatoxylin.—E. Lieb ("Modified Phosphotungstic Acid–Hematoxylin Stain," *Arch. Path.*, 1948, **45**, 559–60). The following staining solution was found satisfactory:

Phosphotungstic acid	..	..	..	..	.. 10 gm.
Hæmatoxylin ..	..	..	..	..	500 mgm.
Red mercuric oxide	..	..	..	..	250–500 mgm.
Hydrogen peroxide	..	..	..	..	.. 2 ml.
Distilled water	..	..	..	..	500 ml.

The hæmatoxylin is dissolved in a small amount of water by heating. The phospho-tungstic acid is dissolved in the rest of the water by heating and then added to the hæmatoxylin solution. The mixture is brought to the boil and the mercuric oxide is added, shaken, and allowed to cool. The hydrogen peroxide is then added and set aside for upwards of a week to cool. The solution should be a deep brownish-red colour. Sections of formalin-fixed material are brought down to water: place for 5 minutes in 0·5 p.c. potassium permanganate; rinse in tap-water; bleach in 2 p.c. oxalic acid for 5 minutes; wash in tap and other distilled water; mordant for 1 hour in 4 p.c. ferric alum; rinse in tap and then in distilled water; stain for from 2 to 24 hours; dehydrate in two changes of absolute *iso*propyl alcohol, clear in xylol and mount in balsam. Staining in the hæmatoxylin is generally complete in 2–3 hours.

G. M. F.

Staining Living Insects.—RUTH V. HERSHBERGER ("Stain Combinations in Living Insects," *Ohio J. Sci.*, 1948, **47**, 161–8). A method is described for staining tissues differentially in living insects. Twelve stains were used to make twenty combinations in normal saline; those used were as follows: Bordeaux Red, Rose Bengal, Biebrich Scarlet, Congo Red, Safranine O, Fuchsin Basic, Blue de Lyon O, Brilliant Cresyl Blue, Azur II, Toluidin Blue, Aniline Blue, and Trypan Blue.

The experimental insect was the American cockroach (*Periplaneta americana*) 6 months old. The insects were anæsthetized with ethyl ether and 0·1 ml. of the stain mixture was injected through the thin cuticle at the base of the third abdominal seg-ment. On injection it was found that a rather slow release of the stain was desirable to prevent injury to the internal organs. The difficulty of the chemical activity of some material upon mixtures which were prone to congeal in a few minutes was overcome by rapid mixing and shaking and the injections prepared before the reaction took place. Much settling occurred in some cases and examination of the solutions showed them to be suspensions rather than true solutions. The author gives a very precise list of her definition of the terms used in the anatomical descriptions. Each stain combina-tion was tested over a maximum period of 2 days, and the examinations made at intervals of ¼ hour, 1 day, and 2 days. A very detailed list is included giving informa-tion of the colouring effects of twenty combinations of dyes upon the various structures with best timing to reveal them differentially. Examinations were carried out with a binocular microscope as well as by unaided vision. F. C. G.

Basic Fuchsin.—E. D. DELAMATER ("Basic Fuchsin as a Nuclear Stain," *Stain Technol.*, 1948, **23**, 161–76, 26 refs.). The conditions under which basic fuchsin acts as a nuclear stain were compared and tested on a Feulgen-weak fungus, *Blastomyces dermatitidis*. Five distinct chemical states were examined, basic fuchsin (the various rosanilines), the Feulgen or Schiff reagent, the Feulgen reagent with aldehyde, basic fuchsin with hydrochloric acid, and basic fuchsin with aldehyde. Aqueous basic fuchsin is an excellent though impermanent stain with which to study the nuclei of fungi. The permanganate method of mordanting tissues was unsatisfactory. Acid hydrolysis and mordanting in 2–4 p.c. formaldehyde before staining with basic fuchsin the staining of nuclei was satisfactory: in cells that were hydrolyzed but not mordanted the nuclei stained clearly and selectively, but faded rapidly in the alcohols. Cells which were not hydrolyzed but were mordanted stained intensely but unselectively. With animal tissues the results are different. A clear-cut nuclear stain is obtained with aqueous basic fuchsin: the cytoplasm does not take up the stain so avidly either in the absence of hydrolysis or when aldehyde mordanting alone is used.

Comparison of crystal violet and basic fuchsin suggests that the mordanting action

of the aldehyde operates through the *para*-amino group of the dye. Gentle acid hydrolysis of the tissues is essential both for the specificity of the basic fuchsin as a nuclear stain and to the mordanting effect of the aldehyde. Fast green FCF is excellent as a counterstain for animal tissues, but not for fungi. G. M. F.

Cytochemical Reactions for Amino Acids.—J. BRACHET, and J. R. SHAVER ("The Effect of Nucleases on Cytochemical Reactions for Amino Acids and on Staining with Acid Dyes," *Stain Technol.*, 1948, **23**, 177–84, 26 refs.). Crystalline ribonuclease has no marked proteolytic activity, for previous digestion of sections with this enzyme produces no appreciable decrease in the intensity of the cytochemical tests for arginine and tyrosine. Cytoplasmic basophilia is unaffected by treatment with cold trichloracetic acid or with boiling alcohol-ether mixture. Mononucleotides and fatty acids thus having nothing to do with basophilia. Digestion of sections with desoxyribonuclease has no effect on the alkaline phosphatase or arginine tests, but it suppresses the Feulgen reaction and the affinity of the chromatin for basic and for some acid dyes. G. M. F.

Staining Cell Walls in Shoot Apexes.—R. A. POPHAM, T. J. JOHNSON, and A. P. CHAN ("Safranin and Anilin Blue with Delafield's Hematoxylin for Staining Cell Walls in Shoot Apexes," *Stain Technol.*, 1948, **23**, 185–90, 6 refs., 2 figs.). Slides are brought to 50 p.c. ethyl alcohol and stained in 1 p.c. safranin O for 24 hours. Dip (raise and lower the slide rapidly 10 or 20 times) in tap-water. Dip in 2 p.c. aqueous tannic acid solution and leave for 2 minutes. Dip in tap-water. Dip in Dellafield's hæmatoxylin and leave for 2 minutes. Dip in tap-water (1 drop concentrated hydrochloric acid in 200 ml. water); drip in 0·5 p.c. lithium carbonate in distilled water and leave for 5 minutes or wash in running alkaline tap-water for 5 or 10 minutes. Dip in ascending strengths of ethyl alcohol and finally in absolute ethyl alcohol. Dip in a saturated solution of anilin blue in " methyl cellosolve " and leave for 5 or 10 minutes. Dip in absolute ethyl alcohol. Dip in a rinse of equal parts of 25 p.c. methyl salicylate, 33 p.c. xylene, and 42 p.c. absolute ethyl alcohol. Dip in a clearing solution (2 parts methyl salicylate, 1 part xylene, 1 part absolute ethyl alcohol) and leave for 10 minutes. Dip in a mixture of 90 p.c. xylene and 10 p.c. absolute ethyl alcohol. Dip successively into two Stender dishes of xylene and leave until ready to mount in " Clarite." Slides should be " cured " for 2 weeks or more in an oven at 40° C. G. M. F.

Radioactive Iodine in the Thyroid.—C. P. LEBLOND, W. L. PERCIVAL, and J. GROSS ("Autographic Localization of Radio-iodine in Stained Sections of Thyroid Gland by coating with Photographic Emulsion," *Proc. Soc. exp. Biol. N.Y.*, 1948, **67**, 74–6). After administration of radioactive iodine to animals the thyroids were removed and fixed in formalin or Bouin's fluid, embedded in paraffin, sectioned 3–5μ, stained, dehydrated, coated with 1 p.c. celloidin, and dried for 6 hours. The photographic emulsion was applied in a dark room: the emulsion of Kodak medium lantern slides was softened by soaking in distilled water for 10 minutes at 19°±1° C. The soft emulsion was placed in a beaker and heated up to 38°–39° C. just before applying the coating. The slides, already stained, were warmed to 38° C. and 4–5 drops of the emulsion were applied over the tissue sections and smoothed with a camel's-hair brush. To make an even coating the slide was tilted from side to side, gelation occurring with the slide on a level surface. Slides were stored in light-tight containers at 0–2° C. and dried over phosphorus pentoxide. Slides were developed at varying intervals; development was for 2 minutes in Kodak D–Z$_2$ at 19°±1° C. and fixation for 10 minutes in acid fixative. The slides were washed for 20 minutes at a temperature

below 20° C. The dehydration of the sections and mounting in Canada balsam are the last two stages. Slides must be dried at room temperature. Short exposures provide better cytological localization of radioactivity.   G. M. F.

Attaching Paper Labels to Slides.—J. B. EBERSOLE (" An Improved Method for Attaching Paper Labels to Slides," *Stain Technol.*, 1948, **23**, 220). To label slides the following technique is recommended. When the mounting medium on the slides has dried the end of the slide is cleaned with a cloth moistened with xylene. Allow the xylene to evaporate or clean the slide again with alcohol. Pipette a few drops of 70 p.c. ethyl alcohol on to the space where the label will be placed. Place the label, gummed with mucilage, directly on the drop of alcohol and press firmly with a clean towel. Label varnish can be added after a short interval, for alcohol evaporates more rapidly than water and few if any " air islands " are left between the label and the slide.   G. M. F.

Clearing Technique.—K. R. SPORNE (" A Note on a Rapid Clearing Technique of Wide Application," *New Phytol.*, 1948, **47**, 290–91, 1 pl., 3 refs.). The use of pure lactic acid (B.P.) instead of lactophenol for the morphological study of a wide range of botanical material is described. The technique is useful for examining the internal vascular structure of flowers, the arrangement of the sporangia within the sori of ferns, and to demonstrate the vascular bundles within the fertile spikes of a number of *Ophioglossaceæ* and to show the arrangement of the sporangia within the cones of species of *Selaginella*. It may be applied to fresh material after suitable dehydration, spirit material, or dried herbarium specimens, and the preparations may be mounted permanently in the clearing fluid. For studying the vascular structure of large or medium-sized flowers, longitudinal and transverse hand-cut slices of tissue up to 2 mm. in thickness may be used, while small flowers may be examined whole. Dried herbarium material is to be soaked out before clearing.

Material should be decolorized with alcohol for chlorophyl lor alkaline hydrogen peroxide (20 vols.) for the brown-coloured dried herbarium material; this latter may also be effected with hot (100° C.) pure lactic acid. Air bubbles may be extracted with a vacuum pump. No staining is required.

Permanent mounts may be made on micro-slides in cells built up and cemented with the following mixture. Shallow cells may be made of the same material:

Lanoline, anhydrous .. .. 4 pts.
Resin, powdered .. .. 8 pts.
Balsam, dry powder .. .. 1 pt.

These are melted together by heating, and the mixture is found resistant to lactic acid. Cover-glasses are sealed with a hot knife, and the preparations should be stored flat.

The plate shows three pairs of stereoscopic photomicrographs of preparations mounted in 1939 of tetramerous flowers of *Sparmannia africana* (*Tiliaceæ*).
                          F. C. G.

Substitutes for Cedar-wood Oil.—C. VAN DUIJN, Jr. (*Microscope*, 1948, **7**, 91–3). Cedar-wood oil has a refractive index between 1·50 and 1·51 (N20/D) and must be thickened to the correct refractive index of 1·515 for permanently covered preparations. Various substitutes may be used. The most important are anisole, methyl benzoate, and methyl salicylate. Anisole is methoxybenzene: the refractive

index N20/D=1·517; it is a colourless liquid with a specific density of 0·992 and a boiling-point of 154° C. The viscosity is relatively low.

Methylbenzoate has a refractive index of 1·526 (N20/D). It is less volatile than anisole, but has a higher specific density and is soluble in ether. Colours of stained sections are brighter with anisole or methyl benzoate than with cedar oil. Methyl salicylate has too high a refractive index, N20/D=1·5363. It is not as fluid as anisole and methyl benzoate and has a penetrating smell which may cause headache.

G. M. F.

Palæobotanical Methods with some Results.—ELISE HOFMANN ("Fossile Gewebe unter dem Mikroskop," *Mikroskopie*, 1947, **2**, Hefte 9–12, 296–313; bibliography and 10 photographs). The paper is a combination of a survey of methods of research in palæobotany with the results of examinations of materials by old and new methods. The bibliography is incomplete; Darrah's work is not mentioned, yet the impression is created that knowledge of work after 1939 has been available. The paper is one of the best and most comprehensive reviews of palæobotanical methods available. In the original, methods and results are given together; for the purpose of this abstract it has been found convenient to separate methods from results.

The epidermal cells of coal plants can be developed by treating the fossil with eau de javelle in concentrations of 1 : 2 to 1 : 3 in a petri dish for times varying from a few hours to several days. The epidermal layer floats off as a white membrane and is mounted in the usual way. Safianin may be used as a stain. Ferns, cryptogams, Ginkos, and conifers can be displayed by maceration. The method of J. Walton is useful in difficult cases. Stone is dissolved away by hydrochloric acid, followed by hydrofluoric acid. The residue may be mounted in any normal manner.

The peel method of Ashley is useful for thick specimens of the permo-carboniferous flora, the section after treatment with hydrochloric and hydrofluoric acids is flooded with a cellulose-acetate–amyl-acetate solution; when this has set the cellulose acetate film is peeled off, bringing with it a film of coal, this is mounted in the usual way.

For spore grains from the tertiary, microincineration is used. The material is ashed by placing it in a hot porcelain evaporating basin. The ash is placed on a microslide, moistened with hydrochloric acid, washed, and mounted.

Maceration followed by incineration reveals epidermal structures such as stomata, hairs, and other specialized structures.

Replica methods should be used when only the cast of the original plant is available. A collodium acetone solution is allowed to flow over the impression, when the dried film is removed and examined under the microscope it will be found to demonstrate detail even to the form of the epidermal cells. One or two casts should be made to clean up the impression, usually the fourth or fifth will be found best for study. Ordinary thin sections of coal plants should be made whenever possible.

Brown coals yield good results with thin section methods; xylite is brought out by the method. The thin section differentiates at once the simple tracheids of the pine from the complicated structure of the deciduous plant. To cut a thin section of coal soak the specimen in water, glycerine, and alcohol, mount on the microtome, play a jet of high-pressure steam both on material and on microtome knife, under these conditions thin sections can be cut.

Pollen analysis, so successful with peat and bog plants, may also be applied to coal because of the persistence of pollen grains and the exine during fossilization. In dealing with peat 1 c.c. of the material is boiled in dilute caustic potash to dissolve the humus; the pollen grains remain. Twelve classes of pollen grains are very specific

under the microscope, *Pinus, Picea, Abies, Corylus, Carpinus, Betula, Alnus, Fagus, Quercus, Ulmus, Tilia*, and *Salix*.

In geological deposits the pollen is separated by treating 10/20 gm. of material (e.g. coal) in a litre flask with 50 c.c. water and 50 c.c. of 60 p.c. nitric acid for 24 hours; oxidation is then slowed by the continuous addition over 24 hours of such small quantities of water that the flask is then full. The disintegrated material is washed by decantation, covered with 7 p.c. caustic soda solution, and again washed. The pollen is separated by centrifuging and dyed with fuschin before mounting. Coordinate graticules are used for making counts. For very hard coals treatment with Schulze's reagent, chromic acid, or diphenol is necessary. Centrifuging in heavy liquids is a valuable method of pollen or spore separation.

The normal methods of microchemical analysis apply to much fossilized material: e.g. grains of millet preserved in the Salzburg salt mines for more than 2000 years still give the reaction for starch.

Microchemical methods have been developed for the identification of cellulose, lignin, cutin, suberin, waxes, and resins in coal. Cellulose gives a blue colour if decomposed with concentrated boiling caustic soda and afterwards treated with iodine sulphuric acid. Cellulose has characteristic colours in polarized light. Lignin gives a clear violet colour with cobalt theocyanate. Cutin is differentiated from cork by its resistance to solution by a glycerine-chromic acid mixture. Resin may be proved, if undecomposed, by boiling in alcohol followed by staining with Sudan III. In all palæontological work it is necessary to proceed from known living structures backwards in time, and so develop knowledge of the structure and function of the various parts of ancient plants. The experimental methods devised for the study of palæobotany are applicable to many other types of investigation. Pollen analysis and maceration will give information about the types of grain used for food by early man. Microchemical analysis and the methods described above will bring exactness to the study of the fibres and textiles used by primitive man.

The variety of plants existing in the coal measures can be studied only by the application of thin section and peel methods. Early copper beech capsules were identified in the Newstiff area of S. Austria by using the peel method. Thin sections of materials from Hausruckes in N. Austria revealed *Taxodioxylon sequoianum* and *Taxodioxylon taxdii*, a fossil form of the cyprus now found in the cyprus swamps of the Atlantic states of North America. Wood and plant remains preserved in their original form have been found in the Saltzberg salt mines. The seed of *Vicea fata* has been found to give the starch reaction test even when 2000 years old. In the opinion of the Author palæobotany has now reached such development that it should be regarded as a fundamental science.   A. E. J. V. and K. V.

Staining Glycogen.—J. VALLANCE-OWEN (" The Histological Demonstration of Glycogen in Necropsy Material," *J. Path. Bact.*, 1948, **60**, 325–7, 11 refs.). Observations were made to find out to what extent the paraffin blocks of formol-fixed material collected at necropsies could be used in an investigation of the glycogen content of the liver. As controls the liver of rabbits was fixed immediately after death in absolute alcohol and Bouin's fluid. The same tissue was similarly fixed in 4 p.c. formaldehyde for 24 and 48 hours and for 4, 8, 16, and 38 days, or exposed to air on the bench at room temperature for 2 hours and then fixed in (a) formaldehyde or (b) Bouin's fluid. The remainder of the liver was meanwhile placed in a jar with a ground-glass stopper and put in the refrigerator at about 3° C. After 18 and 48 hours respectively pieces were fixed in either formaldehyde or Bouin's fluid.

After 24 hours in each fluid the tissue was transferred to 90 p.c. alcohol, thereafter

to three changes of absolute alcohol, 1 hour in each, left in chloroform overnight, and embedded in paraffin wax. Also, after 4 days in formalin, pieces were placed in water for 12 hours before being taken through the graded alcohols and embedded.

Sections of an average thickness of 6 or 7 microns were floated on 70 p.c. alcohol at 55°–60° C., mounted, and dried, and after removal of the paraffin were covered with a film of 1 p.c. celloidin. They were then stained with hæmatoxylin and Best's carmine. Two sections were covered with celloidin and treated with saliva for 15 minutes before staining. These saliva-treated sections were devoid of glycogen. All the others contained large amounts of glycogen in the cytoplasm of the liver parenchyma. The glycogen particles were smaller in the alcohol-fixed tissue than in the tissue fixed by other methods. The glycogen appeared in slightly larger amounts in the tissues fixed in Bouin's fluid for 24 hours and in formalin for 24 and 48 hours. Any formalin-fixed tissues embedded in paraffin can thus be stained for glycogen provided that the sections are cut and mounted in the appropriate way.   G. M. F.

Blue Light Fluorescence Microscopy.—S. STRUGGER (" Fluorescence Microscopic Examination of Trypanosomes in Blood," *Canad. J. Res.*, 1948, **26**, 229–31, 1 pl.). A method is described for *intra-vitam* selective staining of trypanosomes in blood; the method is also applicable to dried smears. Examination is carried out with a blue light fluorescence microscope.

The blue light fluorescence microscope is constructed as follows. The source of light is a carbon arc with a parallelizing lens, and is filtered by a cuvette (2·5 cm. thick) filled with a solution of saturated copper oxide ammonia so that only blue light reaches the mirror of the instrument. The optics are of the usual type, but a filter is fitted over the ocular of an orange glass which absorbs the blue light quantitatively but allows green, yellow, and red light to pass through quite unchanged. To focus the fluorescence microscope a slide on which is placed pulverized anthracene in liquid paraffin is used.

For vital dying as a fluorochrome a solution of acridin orange 1 : 1,000—made with 0·85 p.c. sodium chloride—is used, and a drop of freshly taken blood is mixed with this solution on a slide and covered with a cover-slip. The erythrocytes are non-fluorescent and scarcely visible. Sporadically situated leucocytes are vitally stained with acid in orange, their nuclei and protoplasm have a strong green fluorescence and very accurate counting may be carried out in this way. The trypanosomes shine with bright, light green fluorescence, do not loose their motility, and are easy to find under low power. Cytological examination may be carried out on living trypanosomes under an oil-immersion lens: the cytoplasm fluoresces a diffuse green and the cilia also are stained; the nucleus appears a bright yellow-green and the blenharoplast is clearly visible. The method should be useful for chemotherapeutical research because of the different reaction of living and dead cytoplasm.

For dried blood smears the method followed is to fix after drying for 2–3 minutes in methyl alcohol and, after a short washing, it is stained for 4 minutes in a solution of auramine (1 part dissolved in 1000 parts of distilled water with 5 parts of liquid phenol added). It is then washed in distilled water 1–2 minutes and dried in air. Slides should be kept in the darkness. The image obtained is a black background with the erythrocytes shining brightly as dark green circles. The leucocytes are not visible and the trypanosomes show up in a bright golden fluorescence with much detail visible. Mounting is good in liquid paraffin.   F. C. G.

CYTOLOGY.

Carotenoids in Golgi Apparatus.—A. J. CAIN (" The Accumulation of Caro-
tenoids in the Golgi Apparatus of Neurones of *Helix, Planorbis,* and *Limnœa,*" *Quart.
J. micr. Sci.,* 1948, **89,** 421–8, 4 text-figs.).   An investigation was undertaken to deter-
mine the chemical nature of the coloured granules in neurones of pulminate gastropods.
The work of Thomas is considered wherein the coloured granules in cerebral galglia
of *Helix aspersa* are believed to be a Golgi product.   There is much more in the neurones
of *Limnœa stagnalis* (*L.*) and *Planorbis corneus* (*L.*).   In the former the central nervous
system is distinctly coloured by the pigment, and a certain amount of hæmoglobin
adds to the bright red coloration.   The methods used are given as follows:

(i) For lipids in general, material was fixed in formol-calcium and frozen sections
were cut and coloured with Sudan black B, which is specific for lipids.   It does not
colour pure carotenoids or *solid* lipid.   As carotenoids are soluble in lipid solvents
they are included under the heading of lipids.

(ii) Baker's acid hæmatein test for phospholipines with pyridine extraction as
control.

(iii) For detection of carotenoids the Carr-Price reaction was used.   The blue
coloration obtained in carotenoids and vitamin A using antimony trichloride ($SbCl_3$)
in chloroform is not permanent.   A test was used by exposing the section to the action
of light and air and the rate of fading was noted.

Living cells were observed in sodium-chloride saline and stained supravitally with
neutral red chloride, methylene blue (BDH), Nile blue, and Janus green B (Höchst).
Mann-Kopsch preparations were made of neurones from all the species, and Thomas'
variant of the Mann-Kopsch technique was used with *Planorbis*.   *Helix* neurones were
fixed in Helly, postchromed, stained with Altman's acid fuchsin, differentiated with
sodium carbonate solution, and counterstained with methyl blue.

Under results the author describes a large number of bodies in living *Helix* neurones
either subspherical or irregular, or, as described by another worker, " mulberry "
forms; they are more thickly aggregated towards the axon hillock.   The rim of
the spheroidal bodies show a granular appearance and are often of a yellowish colour.
Methylene blue (1 in 10,000) stained the latter very deeply.   The rims of these bodies
are blackened with osmium vapour.   Filaments and coccoid chains were seen in living
cells and distinct filaments were seen in *Helix* after application of Janus green B
(Höchst); there was an equal distribution of these bodies throughout the cell.   After
6 days, osmication (Mann-Kopsch) in *Helix* " batonettes " were seen in nearly all cells,
this method also showed an aggregation in the hillock.   In *Limnœa* and *Planorbis* the
batonettes were less obvious and the globules more so.   In *Helix* material fixed with
Helly, postchromed, and stained with acid fuchsin and differentiated with sodium
carbonate solution no mitochondria were seen, but spheroidal complexes with very
fuchsinophil rims and clear interna were present.   A varied picture was obtained with
Baker's acid hæmatein test; the cytoplasm a clear yellow brown with blue-stained
bodies or coloured throughout.   It was thought that these cells were damaged and a
lipophanerosis had taken place so that phospholipines were liberated in the cytoplasm.
The Sudan black method after formol-calcium fixation with the material cut on the
freezing microtome provided a picture almost exactly like that of living cells exposed
to osmium vapour.   The yellow or orange-yellow pigment in the cells fades under the
action of light and air, but concentrated sulphuric acid produces a blue-green colora-
tion immediately; other workers concluded that the pigment was " lipochrome."
Iodine in potassium iodide solution gives a deep violet.   This indicates that the
pigment is a carotenoid and is confirmed by the blue colour given with a solution of

antimony thrichloride in chloroform. After 6 hours in formol-calcium the carotenoid-containing granules take up less Sudan black than fat droplets of a similar size, this suggests that the largest granules are compounds of carotenoids only among the lipoid, but this writer considers that the presence of protein cannot be ruled out. It is suggested that the complex contains phospholipids and perhaps other lipids in the rims, caps, or associated granules, and carotenoid in the interna. Vitamin A cannot be excluded, but its presence is not established. F. C. G.

Golgi Material of Drosophila.—W. SIANG HSU (" The Golgi Material in the Salivary Glands of the Larvæ of *Drosophila melanogaster*," *Quart. J. micr. Sci.*, 1948, **89**, 401–14, 26 text-figs.). Emphasis is laid upon the morphology, distribution, and behaviour of the cytoplasmic elements of the cells ; the following facts were found to be of interest : (1) up to the second moult the growth rate of all the cells is apparently uniform throughout the gland ; (2) from the second moult on, the cells in a gland do not all grow at the same rate ; those in the distal portion exhibit a higher rate of growth and are larger than the proximal ones. In the youngest cells observed the Golgi material is distributed at random and does not seem to favour any one particular location. Besides the Golgi elements very minute droplets or vacuoles of about the same size can be seen lying free in the cytoplasm. In older cells the Golgi bodies increase in size together with the size of the cell, although no increase in number of the elements is observed. The lighter internum is now visible and a number of relatively larger vacuoles are to be seen ; the minute droplets lying free in the cytoplasm do not appear to be either of a nucleal or mitochondrial origin. The condition in still larger cells suggests that these droplets are elaborated by the Golgi material and set free in the cytoplasm, the larger vacuoles being formed by their fusion. The condition found in these cells resembles that in the actively secreting cells of the glandular portion of the proventriculus of the larvæ, but in the latter that droplet fusion does not take place. These cells show the lumen ends broken and discharging into the lumen portions of their cytoplasm secretion droplets and vacuoles ; this is an exhibition of the periodic release of digestive enzymes elaborated by the Golgi bodies. As no nucleus of any cell has been seen to be affected by this process of release it is concluded that the cells have the power of repair after discharging their secretion into the lumen. In the next stage of growth a new sort of granule is found : these granules are about the same size as the Golgi bodies, but appear to have a less watery consistency than the digestive droplets ; they grow larger, become more numerous and are considered to be storage granules. They are more numerous at the basal pole of the cell. During cytolysis what is left of the Golgi material does not show any signs of disintegration. Chondriomites are numerous and do not align themselves into threads so often as in younger cells. When the cytoplasmic inclusions are in the lumen of the gland they loose their morphological identity and the individual stainability which they formerly possessed.

Mitochondria appear in the form of chondrioconts and chondriomites and vary in size and appearance ; no evidence has been obtained to attribute the origin of vacuoles and droplets to them. In a later stage there appears among the larger chondriomites some which do not stain so deeply as the rest and appear to stain less deeply as the size increases; they eventually fill up the cell and are transferred to the storage granules. The differential staining of parts of these granules, which appear just before cytolysis, sometimes as coloured crescents or caps according to the stain used is possibly due to uneven transportation of mitochondrial material into storage granules, the coloration denoting a retention of the chemical composition of the mitochondria.

In the discussion the author states that several other workers saw no evidence that either Golgi bodies or mitochondria play a part in secretion synthesis. He calls it Golgi-material-and-Secretion complex; when it reaches a certain size the drop of secretion is released into the cytoplasm, and he finds no indication that secretory material is first separated under the influence of the mitochondria and then moved up to the Golgi material to be matured into secretory droplets. It appears that after performing the offices of secretion of digestive enzymes the cells of the salivary glands take upon themselves another function, that of storage. Interesting details of the functioning of these gland cells during metamorphosis are recorded. F. C. G.

The Seasonal Histological Changes occurring in the Ovary, Corpus Luteum, and Testis of the Viviparous Lizard, *Xantusia vigilis*.—MALCOLM R. MILLER (*Univ. California Publ. Zool.*, 47, No. 8, 197-224; pls. 11-14, 1 text-fig.). This paper is based on a seasonal histological study of the gonads of at least four adult ♂ and four adult ♀ from each of twenty-five collections made over a period of 1 year.

All ovaries were fixed in Susa fixative and preserved in 70 p.c. alcohol; material was embedded in paraffin, sectioned, and stained in Heidenhain's modification of Mallory's stain.

The completely transparent ovarian epithelium permits the individual ova to be seen. The ovary is composed of ovarian epithelium, stroma ovarii, the ovarian cavity, germinal bed, corpora atretica, corpora lutea, and ova. Each ovary produces annually one completely developed ovum. Two ova may develop in one ovary, but in this case the other ovary has no ovum. During maturation of the definitive oocytes about half the intermediate and smaller ova undergo atresia.

The essential developmental and structural features of the corpus luteum is similar throughout the Lacertilia, and the length of life of the corpus luteum in *Xantusia* agrees closely with that of most of the viviparous forms described hitherto. The corpus luteum is formed by hypertrophy of granulosa cells of the ovulated follicle, and full development is not attained until the fourth week of gestation. The significance of the variations in the development and structure of the reptilian corpus luteum is discussed.

Concerning the ovarian cycle, after parturition in September the ovary becomes more active, there being slow growth of ova during the autumn and winter, this being followed in the spring by a rapid laying down of yolk. Final maturation takes place in April and May.

Although no histological study of the oviduct has been carried out on *Xantusia*, the seasonal changes in the mass of the whole oviducts suggest that they may serve as an indicator of ovarian activity. Shortly after parturition in September, the average mass of the oviduct drops from 4·5 to 3 mgm., due to resorption of the gestational epithelium. Other correlations of a similar nature are mentioned.

The ovarian cycle of *Xantusia* corresponds closely to that in *Phrynosoma* and *Hemidactylus*.

The microscopic anatomy of the testis is similar to that described for other lacertian forms. The number of interstitial cells is small and does not exhibit a seasonal cycle. *Xantusia* apparently possesses only five pairs of chromosomes in its nucleus.

The seasonal testicular cycle is similar to the usual Lacertilian type: The first mature sperm begin to pass from the tubules into the epididymus by mid-May, and almost all the tubules have evacuated their spermatozoa by mid-June. The seminiferous tubules collapse shortly after mating. There is a rapid spermatogenesis during the summer and autumn, followed by spermiogenesis in the spring prior to the shedding of the sperm in May or June.

The testicular cycle in *Xantusia* differs from the lacertilian type in the regular and less rapid cytological transformations. A review of the various testicular cycles in Lacertilian forms then follows.

Preliminary experiments by the author on *Xantusia vigilis* indicate that the endocrine relationships in this species are the same as exist in other reptiles and birds. They also indicate that the usual pituitary-gonad and gonad-secondary sex character relationships exist in *Xantusia vigilis*. F.C.G.

The Gross and Microscopic Anatomy of the Pituitary and the Seasonal Changes occurring in the Pars Anterior of the Viviparous Lizard, Xantusia vigilis.—M. R. MILLER (*Univ. California Publ. Zool.*, 47, No. 9, 225–46, pls. 15–17). *Xantusia vigilis* lends itself to the investigation of seasonal histological changes in the pituitary, for being small, it has a minute pituitary gland, while the cells constituting the gland are relatively large, in keeping with the general size of reptilian cells. In addition, the pituitary cells can be differentially stained with ease. *Xantusia* also is viviparous and possesses a well developed corpus luteum during the gestational period.

The studies set forth in this paper were based on material from twenty-five collections spread over a year. The seasonal changes in the pituitary gland of at least four ♂ and four ♀ (adults) from each collection were investigated.

An oblong piece of brain, together with the floor of the brain case, was removed from the rest of the head by a transverse cut at the level of the optic chiasma through the brain and the lower part of the brain case. The sides of this part of the head were then trimmed and the whole placed into a fixative consisting of 9 parts saturated solution of mercuric chloride in physiological salt solution and 1 part neutral formalin. The pituitary being left intact in the sella turcica and protected by surrounding brain tissue, its relation to surrounding structures could be accurately determined and its handling greatly facilitated.

Fixation lasted 8–12 hours, tissues being then washed in distilled water and preserved in 70 p.c. alcohol. Complete decalcification was accomplished in 1–2 p.c. solution of HCl in 70 p.c. alcohol for 24 hours. The entire mid-part of the brain case containing sella and pituitary was either sagitally or transversely sectioned at 5μ, all sections being stained with Heidenhain's Azan and mounted in gum damar.

The relationship of the pituitary in *Xantusia* to surrounding structures is similar to that described in other reptiles.

The gland consists of three lobes characteristic of all vertebrates: the pars anterior, pars intermedia, and pars nervosa, there being no indication of a pars tuberalis. The gland is asymmetrical (differing in this respect from most other reptilian forms), some variations in the extent of the asymmetry being found.

An extensive and definite hypophysial cavity separating the pars intermedia from the pars anterior is present, this not having been described in other reptiles. The hypophysial cavity is formed by a dorsal cap-like projection of the pars anterior, enclosing the pars nervosa and pars intermedia.

The pars anterior is composed of four types of cells: two types of acidophils, a basophil, and a chromophobe, each type tending to be restricted to definite areas of the pars anterior. The basophil and the chromophobe are similar to the general reptilian type.

The first type of acidophil is restricted to the caudal half of the pars anterior and borders on the sinuses and capillaries of the posterior half of the gland, whereas the second type is entirely confined to the cranial half. This latter acidophil is characterized by fine, deeply staining granules. The nucleus is basal, spherical, large, and

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vesicular, containing a prominent, slightly eccentric acidophilic nucleolus. It is pointed out that the staining reaction of the fine granules of the large (second type) acidophils varies from individual to individual, depending in part on the length of time of staining in azocarmine aniline blue. The significance of this staining variability of the predominantly acidophilic cells is not known.

The pars intermedia is composed of a cap of cells completely surrounding the pars nervosa. No capillaries, sinuses, or colloid-filled follicles have ever been observed in the pars intermedia of *Xantusia*.

The pars nervosa is a thin layer of nervous tissue directly overlain by the pars intermedia and distinctly delimited from it by a layer of collagenous fibres. Only rarely were nuclei found in the nervosa substance and no colloid-containing vacuoles, capillaries, or blood vessels were observed.

Emerging from the histological study of the pituitary in *Xantusia* is further support of the fact that considerable differences exist from one reptilian form to another in the nature of the acidophil cells. This is apparent in spite of differing staining techniques which render accurate comparisons difficult. On the other hand, a close similarity exists between the basophil and chromophobe in the four species investigated to date.

The histological structure of the pars intermedia agrees closely with the general picture found in reptiles, although in this species it lacks the colloid-containing masses present in that of *Sceloporus* and *Anolis*. The histological structure of the pars nervosa, while conforming to the general reptilian type in the nature of the fibrous background substance and the presence of a few nuclei, differs in being somewhat more vesicular, in being penetrated by cytoplasmic strands from the infundibular ependymal epithelium, and in being non-vascular.

Investigation of the seasonal histological changes in the pars anterior has shown that seasonal changes occur in the size of the gland, the absolute and relative number of each of the four cell types, the extent of granulation of the chromophilic cells, and in the abundance of mitochondria.

From a series of lucid graphs and tables in the text it is shown that there is a likelihood of testicular and ovarian activity being more closely associated with basophilic activity or an increase in the percentage of basophils in the pars anterior than with any other cell type.

During September, when there is an increase in ovarian activity, acidophils are decreasing in abundance, whereas the basophils increase. Further correlations between ovarian activity and basophilic production are instanced. After ovulation the growth of ova is at a minimum and the basophilic level approaches a minimum. The corpus luteum, if hormonally controlled, may be associated with an increase in acidophil cells.

Xantusia agrees with *Sceloporus* in the parallel between increase in size and abundance of mitochondria and increase in chromophilic activity.

The author concludes that in *Xantusia*, as in many other kinds of vertebrates, the basophil cell is probably most closely associated with reproductive activity and the acidophil cell with growth, bodily activity, and the lutein phase of the gestational state.

E. D. H.

Golgi Apparatus of Sympathetic Neurones.—O. L. Thomas (" A Study of the Spheroid System of Sympathetic Neurones with Special Reference to the Problem of Neurosecretion," *Quart. J. micr. Sci.*, 1948, **89**, 333–50, 6 text-figs., 2 pls.). The conflict of opinion about the precise nature of the Golgi apparatus and the findings of Baker that in the nerve-cell the structure does not take the form of a net-work but

of separate discrete or " dispersed systems " lying in the cytoplasm are discussed. The superior mesenteric ganglion of the mouse was found to be very suitable for high-power observation of living sympathetic neurones. The cytoplasm of the ganglion cell is seen to contain a variable number of small roundish bodies, more or less evenly distributed throughout, and varying in size from small granules to larger compound objects with a yellowish spherical core, to which are attached smaller dark grains or crescentic caps. The largest bodies resemble sometimes the mulberry spheroids of *Helix* neurones. If these are fixed in formol-saline or Bouin's fluid and stained with hæmatoxylin and eosin the cytoplasm appears homogeneous.

Janus black is reported to be very specific for mitochondria to the exclusion of the spherical bodies; the most successful observations were carried out on peripherally situated mitochondria in cells only just penetrated by the dye. Janus green B was found to be unsuitable for mouse cells. Observations were carried out on pieces of mouse ganglia subjected to osmium vapour by the hanging-drop method. More natural appearances as compared with the observations of the living cells were obtained by short periods of osmication by the Mann-Kopsch technique.

Rabbit anterior mesenteric ganglia were chosen for the application of the Sudan-black method of Baker with frozen sections. The formol-calcium Sudan method applied to these cells reveals the presence of small, clear vacuoles scattered throughout the cytoplasm. Each vacuole carries the characteristic Golgi-like periphery. Sympathetic neurones prepared by this method have an appearance which cannot at first sight be reconciled with any of the classical pictures of the neurone. These vacuoles correspond in size and distribution to the spherical bodies observable within the living cell stained with neutral red. The intraneuronal granules are intimately associated with the Golgi spheroids. The smallest granules appear to be found within the lipoidal pellicle of a single Golgi system. This finding is in accord with the observations of Hersch and others that in exocrine gland cells the secretion antecedents always appear first in this situation and emerge later from the pellicle as independent zymogen granules. The Golgi material when in contact with the nucleus resembles some of the stages of nucleolar emission reported in ova during yolk formation in *Saccourrus* and *Diemyctylus*.

Fixation in acetic-osmic-bichromate of Bensley or Helly's fluid is followed by post-chroming at 37° C. with a saturated solution of potassium dichromate. (The dichromate salts conserve the lipids by converting them into alcohol-insoluble substances which remain sudanophil after embedding.) Wash 4–6 hours at the tap. Dehydrate and embed in paraffin. Cut and mount sections on slides. Take slides to 70 p.c. alcohol and leave 3 minutes. Place in saturated Sudan black in 70 p.c. alcohol 7–10 minutes. Quickly rinse in 50 p.c. alcohol to remove surface stain particles. Rinse in water. Counterstain in carmalum. Mount in Farrant's medium.

The cores of the spheroidal bodies appear as faintly yellowish-brown masses and not clear vacuoles, as in the frozen sections. Closely applied to the core are a number of strongly sudanophil granules or crescents. Sometimes there is a more or less complete sudanophil pellicle to the core, but the more general appearance is of attached granules, whereas in the frozen sections crescents predominate. The largest spheroidal bodies often have a large number of dense black granules almost completely covering their surfaces.   F. C. G.

Polyploidy in the Genus Solanum.—M. WESTERGAARD (" The Aspects of Polyploidy in the Genus *Solanum*: III, Seed Production in Autotetraploid and Alloploid Solanums," *Det Klg. Dansk Videnskabernes Selskab Biologiske Meddelelser*, 1948, **18**, Nr. 3, 1–18, 8 tables). The seed production in a number of *Solanum* species and

their polyploid derivatives is considered.   Chromosome doubling was induced by a combination of the callus method and colchicine treatment; details of this are given.

The natural species all belong to the *Morella* group with a basic chromosome number of X=12, seven monobasic species (2n=24), nine dibasic species (2n=48), and seven tribasic species (2n=72).

The monobasic species *S. adventitium* and *S. nitidibaccatum* are unable to cross with others of the group.   Others can be crossed with difficulty, but the hybrids are sterile. *Ss. nodiflorum* x *gracile* produces a few seeds; *Ss. nodiflorum* x *nodiflorum var. dentatum* is entirely fertile.   The dibasic species are very closely related except for *S. retroflexum*, which is rather isolated morphologically, being the only species with black berries, and crosses only with difficulty with the other dibasic species; and the fertility of the hybrids is rather poor.   The remainder of the group cross readily; the hybrids are quite fertile and regular Mendelian segregation are recorded in F_2.   Probably all these species should be considered varieties of *S. villosum*.   The dibasic species can all be crossed to all of the monobasic species except *Ss. adventitium* and *nitidibaccatum*. The hybrids are completely sterile.   The tribasic species are likewise closely related. *S. guineense* and probably *S. Roberti-Eliæ* are somewhat isolated from the *S. nigrum* types.   Hybrids between *Ss. guineense* x *nigrum* show reduced fertility and complicated segregations are recorded in F_2.   Autotetraploids have been produced experimentally from all of the seven monobasic species (2n=48 in the tetraploids), from seven diabasic species (2n=96 in the tetraploids), and from seven tribasic species or varieties (2n=144 in the tetraploid).   Crossing between monobasic species with subsequent chromosome doubling of the hybrid produced six amphidiploids (2n=48). From crossing between monobasic and dibasic species, twenty-four amphidiploids were raised (2n=72).   Chromosome doubling of the hybrids between monobasic and tribasic species resulted in six amphidiploids (2n=96), and crossings between tribasic *S. nigrum* types and the dibasic *S. villosum* and chromosome doubling of the hybrids gave two amphidiploids (2n=120).

The material comprises twenty-one autotetraploids raised from three different chromosome levels and thirty-nine amphidiploids representing four different chromosome levels.   It is shown that in all cases but one of the auto-tetraploids (1 in 20) reduced berry production ensued.   The average berry production of the tetraploids in the monobasic group is 51 p.c. of the diploid species, and the figure is 55 p.c. in the dibasic group and only 32 p.c. in the tribasic group.   There appears to be no correlation between the seed number per berry and the chromosome level of the species.   In the monobasic group the average seed number per berry of the tetraploids is 44 p.c. of the diploids, in the dibasic group 37 p.c., and in the tribasic group 8 p.c.   One species, *S. guineense*, produces a large number of big parthenogenetic berries without any seeds.

In the allopolyploids the F_1 hybrids are completely sterile except for three cases, two of which, *Ss. nodiflorum* x *gracile* and *Ss. nodiflorum var. dentatum* x *gracile*, had a few berries with 6–7 seeds per berry, and the varietal hybrid *Ss. nodiflorum* x *nodiflorum var. dentatum*, which gave 55 seeds per berry.   In the amphidiploids six cases resulted in a higher berry production in the hybrid than one of the parental species and in one case the hybrid produced more berries than either of the parents. The seed number per berry is also generally much lower in the amphidiploids than in the parents, but in two cases the hybrid produced more than one of the. parental species.   The seed production per plant is also much lower in the amphidiploid than in the diploid parental species except in four cases which are all tribasic amphidiploids. The material provides a good opportunity of comparing the effect of autopolyploidy versus allopolyploidy on fertility: the seed production of the amphidiploids can be compared with that of the autotetraploids of both parental species.

Chromosome doubling causes a considerable increase in the weight of the seeds in all cases and is pronounced in some amphidiploids. In the discussion it is pointed out that the cause of the reduced fertility of the polyploids must be discussed in relation to the meiotic behaviour and pollen function in plants. The reduction in the fertility of the experimentally produced autotetraploids is due to a reduction in the berry production as well as to a decreased number of seeds per berry. In the tribasic group only this reduction is strongly correlated with the chromosome number of the diploid species, whereas in the monobasic and dibasic groups there is a great variation in seed production not correlated with chromosome number. The two monobasic species *Ss. adventitium* and *nitidibaccatum* are less fertile than any of the other monobasic or dibasic species, and this difference cannot be explained as due to visible meiotic differences between the different types and must depend upon the genotypic constitution of the diploid than upon chromosome number when this is not too high. Some of the certified amphidiploid hybrids are more fertile than one of the parental species and in all cases the hybrid has resulted from a cross between two diploid species which show a great difference in seed production, due to the sterility of the F_1 hybrids the genetic background cannot be analysed, but it is assumed to be under the control of polygenes.

Other cases present different results and very high seed producers result in sterile amphidiploids. This again depends upon the genotype of the parents and how far the two genomes can harmonize in the hybrid, the meiotic behaviour of the amphidiploids being of secondary importance. Fertility and sterility are explained rather in genetic terms than on a cytological basis.

The author ends with a discussion on the importance of these problems to the practical plant breeder and the significance of the fact that it is possible to produce amphidiploids giving a higher yield than either of the diploid parents.   F. C. G.

Experimental Sex Reversal in the Red Swordtail Hybrid *Xiphophorus-Platypœcilus*.—Sister ANNE BERCHMANS TAYLOR (*Trans. Amer. Micr. Soc.*, 1948, 67, Pt. 2, 155–64, 4 pls.). Adult females of *Xiphophorus-Platypœcilus* hybrids were injected with testosterone propionate in cotton-seed oil at 24-hourly intervals, males being injected with estradiol on the same basis. This method proved unsuitable, however, no fish surviving more than seven injections, with no external change produced. The adoption of sesame oil in place of the cotton-seed oil, however, proved most satisfactory, the fish surviving numerous injections, the females exhibiting external changes after nine injections at 24 hourly intervals and a lapse of 17 days.

It was finally decided to inject the hormone at 48-hourly intervals, this giving the same results as the 24-hourly method, save that the external changes in the female appeared after the sixth injection and a lapse of 16 days. Each fish received $\frac{1}{20}$ c.c. at each injection, both hormones being mixed with 9 parts of oil to 1 of hormone.

Masculinization of the secondary sex characters occurred in 100 p.c. of the treated females, but no complete sex reversal was induced. The ovary shrank considerably and the medulla became atrophied. All ovaries in section showed a great increase in the interstitial cell, these being presumed to be androgen-forming cells.

One treated female, having exhibited reversal of secondary sex characters, was mated with a normal female, and displayed all the normal male behaviourisms.

The estradiol caused no reversal of secondary sex characters in the male, although it did result in the destruction of the spermatophore, and spermatogenesis in the periphery of the testis was temporarily suppressed. Atrophy of the medulla was apparent in sections. It is concluded from the foregoing that secondary sex characters in the male do not necessarily depend on formation of a testis or spermatogenesis.

E. S. H.

PROTOZOA.

Division in Oral Entamœba.—E. R. NOBLE ("Cell Division in *Entamœba gingivalis*," *Univ. Calif. Publ. Zool.*, 1947, **53**, (7), 263–80, 4 pls.). Description of the process of division in the human oral amœba, *Entamœba gingivalis*. Smears made of material obtained from the mouth were fixed with Schaudinn's, Heidenhain's Susa or Gilson-Carnoy solutions, and stained with Heidenhain's iron hæmatoxylin or Harris' alum hæmatoxylin. The Feulgen reaction was employed to reveal the chromosomes. The resting nucleus has a central granular endosome (=karyosome), irregularly beaded peripheral chromatin, and minute (periendosomal) granules between the karyosome and nuclear membrane. Division of the nucleus is mitotic, without dissolution of the membrane. During prophase the nucleus becomes enlarged, then the periendosomal granules are condensed, while the karyosome is broken up and dispersed. The metaphase is indistinct owing to the loss of stainability in the central granular mass. In late anaphase the elongated nucleus contains two groups of chromosomes connected by fibres crossing in the centre. Peripheral chromatin is reduced in anaphase, being restricted to a band in the middle of the nuclear membrane. The nucleus divides into two before the cytoplasm undergoes fission. Chromosomes, apparently numbering five, seem to originate from the periendosomal granules. C. A. H.

New Flagellate from Tapir.—B. HONIGBERG ("The Characteristics of the Flagellate *Monocercomonas verrens* sp.n. from *Tapirus malayanus*," *Univ. Calif. Publ. Zool.*, 1947, **53** (5), 227–36, 4 figs.). In the fæces of *Tapirus malayanus* kept in the San Francisco Zoological Gardens the author found a flagellate of the genus *Monocercomonas* (formerly *Eutrichomastix*). The flagellates in the fæces and in culture were examined in dark field as well as in permanent preparations. Cultures in Ringer-egg-slant medium were kept at 37° C. with subcultures every third day. The material was fixed in Hollande's cupric picroformol for protein-silver (protargol) impregnation and in Schaudinn's fluid for iron-hæmatein staining. The flagellate, which is named *Monocercomonas verrens* sp.n., has three anterior flagella exhibiting synchronized movements and a trailing flagellum with an undulating movement, which arises behind the anterior group of flagella and terminates in a fine filament. The flagella originate from a single blepharoplast complex hidden under a membrane-like pelta, which has hitherto not been described for this genus. The nucleus has an endosome (=karyosome) and numerous chromatin granules. Its membrane is connected with the blepharoplast complex by a rhizoplast. Dorsally from the nucleus there is a parabasal. A slender axostyle runs from the complex to the posterior end of the body, emerging at its end. Food-vacuoles in the cytoplasm may contain bacteria. No evidence of the presence of a cytostome could be detected. C. A. H.

Dislocations in Trichomonads.—H. KIRBY ("Displacement of Structures in Trichomonad Flagellates," *Trans. Amer. Micr. Soc.*, 1947, **66** (3), 274–8, 9 figs.). The author records observations on cultures of Trichomonad flagellates, in which various displacements of the organellæ in relation to the body take place when the flagellates are examined between slide and cover-slip. It was thought that, when subjected to unusual environmental conditions, the behaviour of the flagellates might throw light on certain features of protoplasmic organization. Among the changes observed in Devescovinids the author describes rotational movements of the organellæ and their displacement in relation to the cytoplasm. In *Trichomonas* the undulating membrane and costa repeatedly became detached and moved to the posterior end of the body, from which they returned to the original position. In other cases there was

a shifting of the anterior flagella to different parts of the body. These displacements are illustrated in a number of figures. The changes observed do not support the contention that there is a durable membrane on the surface of the body, but it appears to be fluid and labile. It would also seem that the flagella and other locomotor structures are kept in position by the physical state of the surrounding cytoplasm. In the light of these studies it was possible to interpret the unusual appearance of a flagellate *Diblepharomonas stercoralis*, described from human fæces and possessing two widely separated flagella. In the author's opinion this appearance was due to the displacement of flagella in a normal strain of a biflagellate monad.   C. A. H.

Infusoria from Wild Sheep.—M. Bush, and C. A. Kofoid ("Ciliates from the Sierra Nevada Bighorn, *Ovis canadensis sierræ* Grinnell," *Univ. Calif. Publ. Zool.*, 1948, **53** (6), 237–62, 2 pls.). Description of ciliates found in the stomach contents of a bighorn sheep, *Ovis canadensis sierræ* in California. The material was preserved in formalin and studied in temporary glycerine and water mounts, as well as in chlor-zinc-iodide solution (for skeletal plates). Permanent preparations were stained with Heidenhain's hæmatoxylin (alcoholic method) and Best's carmine. A detailed illustrated description is given of eight new species of Ophryoscolecidæ, *Entodinium nanum*, *E. orbicularis*, *E. protuberans*, *E. truncatum*, *E. montanum*, *E. sierræ*, *E. bicaudatum*, and *Polyplastron californiense*. For comparison of the Ophryoscolecid faunas of wild and domestic sheep, lists are given of the species of this family recorded from various species of the genus *Ovis*.   C. A. H.

Squalorophrya stenostyla, a New Species of Suctoria.—J. Meacham Hamilton, and Theodore Louis Jahn (*Trans. Amer. Micr. Soc.* 1948, **67**, Pt. 2, 206, 1 pl.). This new species of *Squalorophrya* was found attached to the filamentous alga *Basicladia crassa* which occurred on turtles from West Okoboji lake, as recorded in the abstract of Hamilton's paper on this algæ (see Algæ : Chlorophyceæ). The previously described *S. macrostyla* Goodrich and Jahn was also observed in the same algæ as the new species.

S. stenostyla differs from *S. macrostyla* chiefly in the much thinner lorica, and in this being quite distinct from the stalk. Reproduction was not observed.   E. D. H.

ROTIFERA.

Rotatoria from Massachusetts.—W. T. Edmondson ("Rotatoria from Penikese Island, Massachusetts, with a Description of *Ptygura agassizi* n.sp.," *Biol. Bull.*, June, 1948, **94**, No. 3, 169–75, 263–6; 1 text-fig.). This paper is based on material collected from three main ponds on Penikese Island on August 3rd and 17th, 1947. All three ponds were small and shallow, one being almost dry and nearly fresh (salinity 5 p.c.), whereas the other two contained considerable concentrations of salt. The salinity of the latter two ponds was 34 p.c. and 15 p.c. respectively.

Only one rotifer, namely, *Brachionus plicatilis* Müller, was found in the highest concentration, while the 15 p.c. water contained six species, including one new to science. The rotifers found in this pond were namely, *Collotheca ornata* (Ehrenberg), *Colurella colurus* (Ehr), *Lecane closterocerca* (Schmarda), *Lecane grandis* (Murray), *Ptygura agassizi*, n.sp., and *Ptygura crystallina* (Ehr.). The first pond (of 5 p.c. salinity) contained four species, namely, *Brachionus calyciflorus* Pallas, *Cephalodella catellina* (Müller), *Colurella obtusa* (Gosse), and *Lecane bulla* (Gosse).

All the species are widely distributed and have been previously reported from salt water, with the exception of the two species of *Ptygura*.

The new species of *Ptygura* is described and figured in detail. It resembles in a general way *P. melicerta*, particularly var. *mucicola*. It differs in the shape of the dorsal spine and the lack of any other structures corresponding to the dorsal antenna. The details of the shape of the corona and buccal area are also different, particularly in the angle of the plane of the corona to the axis of the body. Furthermore, *P. agassizi* is not subject to the rigorous substrate limitations of *P. melicerta*.

Special Note.—The moss-dwelling Bdelloids of Penikese Island have been dealt with by André Burger, see Abstract of "Studies on the Moss-dwelling Bdelloids of Eastern Massachusetts" below.   E. D. H.

Ecological Applications of Lansing's Physiological Work on Longevity in Rotatoria.

—W. T. EDMONDSON (*Science*, August 6th, 1948, **108**, No. 2, 797, 123–6). The writer draws attention to the fact that it is becoming increasingly realized that studies of productivity must deal not only with the size of populations at various times but also with rates at which they increase and decline as an expression of the shifting balance between reproduction and death. One of the basic needs in such studies is information about the factors which, in nature, are responsible for shifting this balance.

The writer then reviews some important aspects of Lansing's previously published researches on ageing in rotifers, pointing out that some of the data can be used in interpreting observations on natural populations. This is followed by mention of several important problems arising out of Lansing's work on which further information is desirable, namely:

(i) Whether alkaline species have a higher optimum calcium concentration than others, or whether they are responding chiefly to the pH within the limits of calcium found in nature;

(ii) whether periodic changes of any sort that happen in natural environments have the same effect as treatment with citrate;

(iii) the effect of temperature on the seasonal cycles of many species.

Mention is also made of the fact that chemical factors modify the effects of temperature, and the writer points out that there is reason to believe that interaction of temperature and salinity affects the distribution of several species which tolerate wide variation in salinity from fresh water to coastal salt water. *Notholca striata* var. *acuminata*, for example, is found in freshwater only in the late winter and early spring, when the water is cold, but it occurs all summer in coastal bays and ponds where the water becomes quite warm. It may be expected that survival at high temperatures will be enhanced by increasing the salt content of the medium.

Abstracter's Note: Edmondson gives *Notholca acuminata*, but this is generally regarded as a variety of *Notholca striata* Müller (i.e. var. *acuminata* Ehrenberg).   E. D. H.

Studies on the Moss-dwelling Bdelloids of Eastern Massachusetts.

—ANDRÉ BURGER (*Trans. Amer. Micr. Soc.*, **67**, Pt. 2, 111–42). The North American moss-dwelling Bdelloids, compared with those of other countries, are very incompletely known. The present author examined mosses and lichens from nineteen different localities in the neighbourhood of Cambridge, Mass., and Woods Hole, recording a total of thirty-five species and four varieties. Of these, twelve are new records for the North American fauna, one of which is a species new to science, namely *Mniobia edmondsoni*. A new variety of *Mniobia animosa*, var. *macrocephala*, is also described.

In addition to the above, an unidentified species of *Macrotrachela*, probably but a variety of *M. nana* was observed.

Bryce's definition of the family *Habrotrochidæ* is criticized as being based on a misconception of the nature of the stomach and its physiology. The present author has made a detailed anatomical study of the posterior digestive system in *Habrotrocha ligula*, var. *aligula*. The stomach is a voluminous mass of protoplasm, with twelve to fifteen nuclei, but without any cell walls. The œsophagus is ciliated, the action of the cilia forming a vacuole in the protoplasm at the base of the œsophagus. Into this vacuole food particles are pushed and agglutinated. On reaching a certain size, the vacuole is engulfed in the protoplasm, whereupon another is formed in its place almost immediately. These vacuoles (which are really the " food pellets " of Bryce) develop firm walls, and are moved about the stomach by the churning action of the protoplasm. Matter is digested from these vacuoles, which pass into the bag-like intestine, and are actually ejected by the contractile vesicle, the two being connected by a circular opening. The products of digestion also appear as vacuoles in the stomach, being of various sizes, and when required by the body, nourishment is removed from them; they are then left quite transparent, after which they are ejected in a like manner to the food vacuoles.

An analysis of the distribution data of the species recorded shows that in mosses where the number of species was largest, actual specimens were few, whereas in mosses containing a large number of individuals, the number of species present was quite small.

Lichens from stone walls on Penikese Island harboured a fauna rich in individuals, whereas the moss fauna on this island (which is bare of trees and exposed to violent, dessicating winds) is poor both in species and individuals. The author concludes that under certain climatic conditions lichens constitute a habitat generally superior to mosses.

The specific characters of Bdelloids, the distribution of the Bdelloids collected for the present paper, the physical properties of the various habitats, and methods of culturing Bdelloids in the laboratory, are all dealt with in this paper. Under the latter heading the author gives two interesting facts, viz. that (*a*) under the same conditions, some species are far more prolific than others, and give successful and rapid cultures (a variety of *Macrotrachela concinna* is instanced in example of the latter); and that (*b*) the addition of small pieces of broken glass to the cultures provokes a high rate of reproduction in *Habrotocha ligula* var. *aligula*. Without the glass, there was no reproduction at all in the same fluid and with the same food. The author supposes that the reason for this lies in the fact that the rotifer lays its eggs between surfaces very close together. E. D. H.

Two New Species of Rotatoria from Sand Beaches.—W. T. EDMONDSON (" Two New Species of Rotatoria from Sand Beaches, with a Note on *Collotheca wiszniewskii*," *Trans. Amer. Micr. Soc.*, **67**, Pt. 2, 149–52, figs. 1–14). The material for this paper was collected in 1942 by Dr. J. K. Neel during his ecological study of the interstitial microfauna in sand beaches at Douglas Lake, Michigan.

The two new species are: *Trichocerca neeli* and *Proales longidactyla*. The former resembles members of a group of highly asymmetric species with twisted bodies, including *T. insignis* (Herrick), *T. tortuosa* (Myers), *T. myersi* (Haur), *T. insulana* (Hauer), and *T. eudonta* (Hauer). From the first three *T. neeli* differs in having but one spine on the head sheath. From *T. insulana* it differs in having very prominent dorsal folds on the head sheath, in the absence of prominent styles at the base of the toes, and in the greater size of the left manubrium. It resembles *T. eudonta* most

closely in the head sheath, but *T. neeli* has a less prominent spine and the folds appear different, as also are the toes.

Proales longidactyla differs from all other species of the genus in the great length of the toes and in details of the trophi. It occurred in samples taken 20 cm. from the shoreline, and 1 cm. deep in the sand. The samples were treated with a saturated solution of menthol before preservation. This caused most specimens to be completely extended.

A contracted specimen thought to be *Collotheca wiszniewskii* Varga was found at a depth of 1 cm., 50 cm. above the shoreline. The species was originally described by Varga in 1938 attached to sand grains in the beaches of Lake Balaton in Hungary, and it has not been observed since. E. D. H.

On the Bohemian Species of the Genus *Pedalia* Barrois.—Dr. EMANUEL BARTOS (*Hydrobiologia. Acta Hydrobiologica, Limnologica et Protistologica*, August, 1948, **1**, No. 1, 63–77. The author hastens to point out that most previous workers have been content to label all observed *Pedalia* as *P. mira* (Hudson) (late *Pedalion mirum*, Hudson), without ever bothering to examine their specimens more closely. It is highly probable, therefore, that many records for this species really refer to the other species of this genus.

Species of the genus *Pedalia* may be arranged in four main groups, the *P. mira* group, the *P. propinqua* group, the *P. intermedia* group, and the *P. fennica* group, which together total ten species.

The specific characteristics which it is essential to study closely before identification is arrived at are: (1) presence or absence of dorsal appendages on the posterior of the body; (2) presence or absence of lower lip; (3) dental formula; (4) single or paired insertion of setæ of ventral arm; (5) filamentous or spined development of all arms; and (6) number of spines and/or filaments on each arm.

Three new species are described and figured, namely *Pedalia reducens, P. propinqua*, and *P. mollis*. Extensive keys, tables, and other relevant data concerning the whole genus are given. E. D. M.

ALGÆ AND DIATOMS.

The Marine Algæ of Denmark.—L. K. ROSENVINGE, and S. LUND ("The Marine Algæ of Denmark. Contributions to their Natural History: Vol. 2, Phæophyceæ; 3, Encœliaceæ, Myriotrichiaccæ, Giraudiaceæ, Striariaceæ, Dictyosiphonaceæ, Chordaceæ, and Laminariaceæ," *Det Kgl. Danske Videnskabernes Selskab, Biol. Skr.*, 1947, **4**, 5). A description is given of forms found in coastal waters, an account of their distribution, and a discussion of their taxonomic status. Special attention given to alternation of sporophytic and gametophytic generations; and, in the Laminariaceæ, the change of blade. F. G.

Some Marine Algæ from Mauritius, an Additional List of Species to Part 1, Chlorophyceæ.—F. BORGESEN (*Det Kgl. Danske Videnskabenes Selskab, Biologiske Meddelelser*, 1946, **20**, Nr. 6). An account of findings on material of F. Jadin and Dr. Vaughen examined subsequent to publication of "Chlorophyceæ" as Part I of Borgesen's "Marine Algæ of Mauritius." Important points of the communication are:

New species:

   (1) *Pringsheimiella mauritiana.*
   (2) *Codium mauritianum.*

Changes of name:

(1) *Cladophora fracta*, Küntz, *f. marina* Hauck, ≡ *C. Leteronema*, Brand, becomes for Borgesen, *C. Hauckii*.

(2) *Cladophora composita* (Harv.) of Jadin, ≡ *Boodlea siamensis*, Reinbold, becomes for Borgesen, *B. composita* (Harv.) Brand.

(3) A specimen he listed in " Chlorophyceæ," Part I, as *Codium elongatum* should be referred to a new species, to which he does not care to assign a name at present.

Interesting distribution:

(1) *Ernodesmis veticillata*, and

(2) *Siphonocladus tropicus*
both " West Indian " forms.

F. G.

Algæ from Kamtchatka.—Johs. Boye Petersen (" Algæ collected by Eric Hulten on the Swedish Kamtchatka Expedition, 1920–22, especially from Hot Springs," *Det Kgl. Danske Viderskabernes Selskab*, Biologiske Meddelelser, 1946, **20**, Nr. 1). The author finds that the algæ found by Hulten in the " hot " springs (the quotation marks are to draw attention to absence from the text of a definition of " hot spring ") can be arranged in a series running parallel to one of chloride content of the water, and concludes, with too little reserve, I feel, that wherever those algæ that are found in brackish water at ordinary temperatures are found in hot springs the reason for their presence is a high content of chloride in the water and not the high temperature.

Tables of algæ listed with regard to the maximum water temperature at which they were found—though, as the author states, to be interpreted with reservations—show fairly close agreement with those of other observers. Cyanophyceæ as a group presumably withstand higher temperatures than do the Chlorophyceæ, and the Chlorophyceæ higher than do the Diatomaceæ. An obvious caution is that a natural temperature alga distribution is not necessarily the same as the range of temperature toleration.

Discussing whether there is a special thermal community of diatoms the author concludes that there seems to be no species entirely restricted to hot springs and that those most common in hot springs are probably eurythermal forms selected from the other diatom flora of the locality or country. F. G.

A New Limnological Method for the Investigation of Thin-layered Epilithic Communities.—R. Margalef (*Trans. Amer. Micr. Soc.*, 1948, **67**, Pt. 2, 153–4). The several difficulties encountered in the study of thin-layered algal communities have previously resulted in a number of attempts to overcome them, but none of these, according to the present author, are entirely satisfactory.

He recommends that little stones, fragments of rocks, etc., preserved in formalin solution, be stained with Delafield's hæmatoxyline. They are then washed, passed through a series of alcohols, being finally transferred to absolute ethyl alcohol mixed with ether, the whole process taking half a day. The stones being removed from the alcohol and ether, a small quantity of collodium dissolved in alcohol and ether is dropped upon them immediately, and after this they are allowed to dry. The solidified collodium film thus formed, and containing the epilithic vegetation, is then peeled off the stone and removed to a slide, and cover-glass applied. In good dehydrated

material the films may be mounted in Canada balsam. The author recommends, however, that balsam be avoided and the films mounted in one of the gums commonly used by entomologists (Hayer's solution for example).　　　　　　E. D. H.

Sexual Reproduction in the Genus Basicladia.—JOHN MEACHAM HAMILTON (*Trans. Amer. Micr. Soc.*, 1948, **67**, Pt. 2, 201–05, 1 pl.). *Basicladia chelonum* (Collins) and *B. crassa* (Hoffmann & Tilden) both occur on the Snapping Turtle and the Western Painted Turtle in West Okoboji lake, Iowa State. Both species of *Basicladia* may occur on the same turtle, and so for the purpose of studying the alga, turtles were kept in the laboratory in cement tanks, shielded from direct sunlight. Gametes were sometimes formed within a few days of bringing the turtles into the laboratory. At other times they appeared on slides which had been prepared for 12 hours or longer. Motile cells were discharged in greatest numbers between 9 and 10 a.m., but were observed as late as 10 p.m. These gametes are biflagellate and spindle-shaped, and escape from the mother cell by a lateral pore. The discharge takes place with some force, almost all motile cells having left the mother cell within a minute. In *B. crassa* the gametes fan out from the pore in a stream, but in *B. chelonum* the pore is smaller than the gametes, which have to squeeze through it. Sometimes gametes remain behind in the mother cell after the rest have escaped, these being termed " captive cells," and these are thought to occur in nature.

The gametes fuse to form a spindle-shaped zygote which swims for a period that varies from 10 minutes to ½ hour. After the zygote has come to rest, they lose their flagella and become spherical, after which the chloroplasts appear to fuse and form a mass of chlorophyll.

The author concludes that *B. crassa* is homothallic.　　　　　　E. S. H.

REVIEWS.

Bacteriology: A Textbook of Microorganisms.—FRED WILBUR TANNER, and FRED WILBUR TANNER, Jr. Fourth Edition. 1948. New York: John Wiley & Sons, Inc.; London, Chapman & Hall, Ltd. x+625 pp. Price 27s. net.

Text-books which deal with particular aspects of bacteriology either in an elementary or exhaustive manner are numerous, perhaps too numerous. There is now no lack of authoritative works on medical and veterinary bacteriology or on the microbiology of water, soil, and food products ; however, there are few books which attempt to consider the science of bacteriology as a whole. In this, the fourth edition of a work originally published by the senior author in 1928, a comprehensive account is given of bacteria for all those who are studying microbiology for the first time—with the object, it is stated, of allowing " the student to build his structure on a broad biological basis and to consider only fundamental principles and facts." These claims are very fully met, for there are few aspects, from the early history of bacteriology to the electron microscope, which are not mentioned. There are some specially valuable features. At the end of each chapter is a list of text-books for further reading dealing with the particular subjects discussed. At the end of the book there are an extensive glossary of bacteriological terms and a list of bacteriological literature, including text-books, primary journals, and abstract journals. Some of the text-books listed are now largely of historical interest: Lafar's " Handbuch der technischen Mykologie," published from 1904 to 1914, and Besson's " Practical Bacteriology," published in 1913, are now rarely read. Whereas works in the Penguin series find a place, the two important volumes of Doerr's " Handbuch der Virusforschung " (1939) are unmentioned.

Technical bacteriological details are not recorded. It is obvious that in such a work many subjects must necessarily be dealt with in a somewhat summary manner. Immunity in virus diseases, for instance, is dismissed in eight and a half lines. More serious is the evidence of hurried proof-reading. The legends beneath the photographs of Pasteur and Koch on p. 17, have been interchanged and Carlos Juan Finlay spelt his name without the intrusive " d."   G. M. F.

Essentials of General Cytology.—R. A. R. GRESSON and HELENA HESLOP CLARK. 1948. ix+184 pp.; 71 text-figs. and pls. Edinburgh Univ. Press. Price 21s. net.

The preface indicates that the work is intended for students and teachers in biology in colleges and schools. The introduction, Chapter I, discusses types of cells and their relationship to the tissues they create ; a brief history of cytology comprises the latter half of this part. Chapter II deals with great brevity with the chemical and physical characters of the protoplasm. It is stated that certain modern workers *believe* that protoplasm possesses a microscopic fibrous structure: this is a peculiar way of describing what was, in the year the work actually dates from, to judge by the

references, rather more than a mere case of belief among most workers. Chapters III and IV treat of the structure of the animal and vegetable cell respectively, and quite valuable comparisons are drawn between these two classes of material. Chapters V and VI deal with mitosis, cell division and meiosis. These two chapters are clearly illustrated with good diagrams; the photomicrographs are a valuable adjunct and do not suffer from overmagnification; chiasma formation and terminalization are well treated with good drawings. Different types of fertilization and the cytoplasmic components during maturation and fertilization are considered in Chapter VII. A number of useful historical references concerning the sperm middle piece in the eggs of mammals and the fate of the sperm mitochondria and Golgi material contained therein are noted. Parthenogenesis and the origin of primitive germ cells are described.

Chapters IX, X, and XI deal with reproduction in Thallophyta, Bryophyta, Pterodophyta, and Spermophyta, and it is here, particularly, that the book might prove inadequate, owing to a rather condensed treatment of the subject. Additional words of explanation are needed to appreciate the content fully. Many passages have a somewhat negative value, for, although quite intelligible to a professional, they would never be understood by the intelligent student attending a lecture, but would require a certain amount of elaboration or example. The Chromosomes and Heredity and the Chromosomes and Evolution form the subjects of Chapters XII and XIII. The relationship between characters and structural changes in chromosomes and the part played by mutations in evolution is explained with clarity. Chapter XIV, on the Cytoplasm and Heredity—scarcely four pages in extent, and like most of the book too short—usefully serves to indicate the general tendency away from the exclusive study of the nucleus. In Chapters XV and XVI the Golgi material and mitochondria are considered in a balanced manner, and the authors give the impression of treading surer ground. The diagrams are good but large, and there is a painful lack of photomicrographs accompanying this section. Two brief chapters on the Cytology of the Protozoa and on Degenerating and Pathological Animal Cells end the main part of the work. An introduction to cytological technique does not, beyond a bare statement, enjoin the student to handle his microscope correctly—a technique around which, in reality, the whole success or failure of cytological work hinges. We have yet to find a student's text-book which gives adequate reference to this all-important aspect. A useful glossary of terms deals with modern usage of the words ; the references are classified under chapters.

The book should serve to indicate lines for study to both teachers and their students, and, on the whole, reveals a healthy tendency to treat of the cell as a whole rather than from the point of view of the nucleus and chromosomes; this method should be copied more often. The photomicrographs are of a high order and the book would profit from more of them, particularly in several chapters. Some of the drawings are over large, while others are too diagrammatic : it is undesirable to inflict upon the student diagrams which suggest that structures are more easily seen and found than they really are ; some students are inclined to draw what they think they ought to see. Illustration No. 3, a generalized animal cell, provides an illusion of three dimensions which could, with much profit, be far more common than at present. The book is well printed on good paper with ample margins.   F. C. G.

Dictionary of Genetics.—R. L. KNIGHT. With 9 appendices and bibliography. 1948. Pp. 152+11. Chronica Botanica Co., Waltham, Mass., U.S.A. Price 4 dollars, 50 cents net.

This work includes all those terms which are used in animal, human, and plant genetics; it should prove of very great value to students. The author attempts to

regularize, or, as he states in the preface, " to define and standardize the terminology of the subjects treated," and while not omitting the less used and less suitable terms, emphasizes those which are most apt. Antonyms are dealt with in some cases but omitted in others, " Allotetraploid " and " Autotetraploid " include cross-references, but " Arrhenotoky " and " Thelytoky " do not. The terms " pin-eyed " and " thrum-eyed " may not be so well known in other countries as in England and America and cross-references could with profit be included. Some unnecessary antonyms are included : thus the antonym to " Short-day plants " is given as " Long-day plants." In some cases adjectival forms are printed, such as " Asyndesis," adj. " Asyndetic " and " Apomixis," adj. " Apomictic." The author states that the centrosome does not stain, but in reality it will stain: it would have been better to state that it is not easy to stain differentially. The preface informs us that older terms are included, but, among others, the word " reticulum " is omitted. Some of the entries suffer from brevity and could, without the work assuming the proportions of an encyclopedia, be expanded with profit; examples of this appear under the headings of Golgi apparatus, Zygotic lethal, Amphidiploid and Heterochromosomes.

A number of useful mathematical appendices are placed at the end of the book, one being the international rules for symbolizing genes and chromosome aberrations, and another a list of distances which must be avoided to prevent seed contamination in fertilization. There is a bibliography of works and publications used in compiling the dictionary. The printing is clear, but as the book is likely to be one which will be subjected to much consultation its contents are perhaps worthy of more substantial binding. F. C. G.

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XII.—MITOSIS IN LIVING CELL OF AMPHIBIAN TISSUE   **576.353**
CULTURES.

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Three Plates, One Table and Five Text-Figures.

Introduction.

This paper is an account of observations on living amphibian cells in tissue
culture, mainly with reference to cell division.

In the past, amphibian tissues have largely been used for the study of living
cells in division. As early as 1879, Flemming observed epithelial cells in mitosis
in the tail of the salamander larva. This work formed an important part of
the studies which enabled him to give a definite description of the process which
he named indirect cell-division. To study living cells under the microscope,
it is generally necessary to isolate either a thin layer of tissue, as Flemming was
able to do with the tail fin of the salamander larva, or to prepare a suspension
of separate cells which will continue to live under the conditions of observation.

Jolly in 1901 made preparations of this second type, which contained dividing
cells, by taking blood from newts, under conditions where erythroblasts in
mitosis occur free in the circulation. Jolly discovered that this could be brought
about by feeding newts after a long fast ; he used this method for a series of

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studies on cell division : as, for example, on the influence on temperature on the duration of the stages of mitosis and on the effect of pressure on cell cleavage (1902).

In 1918 he collaborated with Comandon, and together they began ciné-micrographic studies on this material.; they have described the progress of this type of work in subsequent papers (1917, 1934). Their film on the division of newt erythroblasts is of great value.

Tissue culture also is a general method for the study of living cells ; but so far its use for the observation of dividing cells has been confined almost entirely to those of warm-blooded animals. The development of methods for amphibian tissue culture by one of us (M. P.) has already been described (1949). In this paper we present a preliminary study based on cinema records of amphibian cells in division, photographed by phase-contrast microscopy. It is desirable to extend this work in several directions, one of which should be a detailed comparison of dividing cells in life and after fixation and staining. Because such studies have not yet been made, we have not in the present paper considered our results in relation to the previous work on amphibian mitosis in which classical cytological methods have been used.

In the present work, M. P. was responsible for the preparation of the tissue cultures and A. H. for the cinematography. The analysis of the resulting ciné records is the work of both authors.

METHODS.

The culture methods for amphibian tissue which we have used are described in a previous paper (Preston, 1949). The photographic methods were the same as those employed in earlier studies (Hughes and Fell, 1949) and are described in a separate paper (Hughes, 1949).

For the ciné-photomicrography a high-contrast 16-mm. film was used, and the illustrations in pls. I and II are enlargements from 16-mm. negatives. They by no means represent the best results which can be obtained by these means.

MITOCHONDRIAL MOVEMENT IN INTERMITOTIC CELLS OF *Xenopus Lævis*.

Fibroblastic cells at the margin of the outgrowth from explants of connective tissues of the larval limbs of *Xenopus lævis* become greatly expanded and attain a length of 100 microns, or more. In such cells the mitochondria are very conspicuous. Their movements can be appreciated by direct observation ; when speeded up in a " time-lapse " film it is seen to be of very great complexity. In pl. I, fig. 1, a series of stills from such a film record are reproduced. The following types of changes in form and position of these mitochondria are seen :

(a) A reversible change of elongated threads into globules. The globules are conspicuous dark objects by phase-contrast illumination. There are present even larger masses of apparently the same type of material, which is presumably lipoprotein in nature. These large bodies seem stable in

form and do not appear to break down into filaments. However, the fact that the smaller globules can become filamentous indicates that in these cells rounding of mitochondria does not necessarily indicate that the cell is unhealthy. A cell may undergo a normal mitosis with the cytoplasmic inclusions largely in this form.

(*b*) A mitochondrial filament may curl up into a loop, which begins to rotate. Several revolutions are completed; then the filament straightens out once more.

(*c*) Often, filaments move towards the periphery of the cell, with some expansion of the anterior end. Then the tip bifurcates, and two branches separate to either side, so that a tri-radiate mitochondrium is formed which persists for a short time. Finally, by one of various ways, a straight filament is formed once more.

All of these types of changes occurring successively in one mitochondrium are illustrated in pl. I, fig. 1. The incessant variability in form of these cell inclusions suggests that they are fluid rather than solid structures. Possibly they are of the nature of coacervates.

In the fibroblasts of the frog which we have studied, the mitochondria are all filamentous, and their movements are longitudinal.

In the fibroblasts of the newt liver the cell inclusions are all rounded granules, apparently lipoidal in nature.

MITOSIS.

The Newt.

The earliest stage of any mitosis which we followed was in a liver fibroblast of the newt (pl. III, fig. 4). The record of this division begins at a stage when the nucleoli have disappeared, but the chromosomes are not yet fully formed. Their outline is not yet regular; they have a granular structure, and their contrast with the nuclear sap is comparatively low (pl. III, fig. 4 *a*). Gradually, they develop into mature chromosomes with regular outlines (pl. III, fig. 4 *b*). At the end of prophase their appearance suggests a double structure, with some variation in contrast and density along their length. Their width is about 1·3–1·5 microns.

Next, the chromosomes are re-arranged in position and assume a radial formation round a central space (pl. III, fig. 4 *c*). The chromosomes are now V-shaped, with the apex of the V facing inwards.

The radial stage, as this has been called by Hughes and Fell (1948), is the most favourable for study of the chromosomes in cultivated fibroblasts in division. In the newt, the diploid number of chromosomes is 24 (Wickbom, 1945). Comparison of Wickbom's diagram (his Scheme I) with our pl. III, fig. 4 *c*, suggests that nearly all the chromosomes are in focus in this picture, although it is not possible to identify the individual chromosomes in a photomicrograph at a single plane of focus.

The next event in mitosis is that the whole cell and its contents contract (pl. III, fig. 4 *d*) and the plane of the chromosomes rotates through a right angle. This movement takes about 15 minutes. This picture shows the metaphase

plate is side view, no longer in a single plane. At this stage, we see only a single optical section through the metaphase plate, which includes only a small part of the whole structure.

Early in anaphase (pl. III, fig. 4 *e–f*), it is possible to see that the median point of attachment is the first part of the chromosome to move, and that the centrosomes of each group of daughter chromosomes take up a regular formation, first in a plane surface at right angles to the axis of the spindle. This surface gradually becomes more and more concave towards the spindle poles, until at the end of anaphase the daughter chromosomes all converge radially upon the centrosomes. In pl. III, fig. 4 *g*, we have a somewhat oblique view of the spindle. The convergence of the chromosomes upon the spindle poles is better seen in pl. I, fig. 3 *c*. In the chick there is often a curvature of the daughter chromosome groups in the opposite direction (Hughes and Swann, 1948).

In the newt, the chromosomes shorten and thicken during anaphase and apparently become denser. At the end of anaphase those in focus in pl. III, fig. 4 *g*, are about 1·8 microns in thickness. Later, they pass through a stage of apparent fusion.

The cleavage of the cell is initiated by a constriction of the interzonal region of the spindle (pl. III, fig. 4 *g*) and a penetration into it of cell inclusions. A faint longitudinal striation of the spindle is seen at this stage in the newt.

During late anaphase and cleavage there is " bubbling " at the cell surface, variable in extent from cell to cell. This process resembles that seen in dividing cells of higher vertebrates in tissue culture ; cytoplasm flows out beyond the cell boundary, then the flow stops, and is resumed in the reverse direction until the bubble is withdrawn. There is a tendency for bubbles again to be extruded at the site of previous ones.

" Bubbling " gradually ceases as cleavage is completed and the daughter cells begin to flatten. By this time the groups of daughter chromosomes have begun to develop into the nuclei of the two cells. In the newt the most striking change is that soon after cleavage the chromosomes lose their regular form (pl. III, fig. 4 *g*). They pass through a stage of apparent fusion and later become beaded once more. The denser segments seem to extend laterally and give a brush-like border to the chromosome. Gradually the contrast of the chromosomes fades, although some granules persist to form the chromocentres of the resting nucleus. A number of small nucleoli gradually develop.

Mitosis in Xenopus *and* Rana.

In the earliest stages of mitosis in *Xenopus* which we have so far recorded with the ×95 objective, the chromosomes were radially arranged when photography began. Here, the transition to the stage of the transverse metaphase takes place by a re-arrangement of the chromosomes in the transverse plane rather than by a rotation of the whole radial system. In the frog the corresponding stages have been followed so far only with a ×40 objective ; here, the whole arrangement rotates, as in the newt.

In *Xenopus* the contraction of the cell from its extremely flattened intermitotic form to the partially rounded stage of metaphase is continuous from the

radial stage onwards. The expanded cell processes flow centripetally, leaving behind extremely fine propoplasmic filaments, radiating from the rounded cell.

In *Xenopus* and *Rana* at metaphase the clear mitotic spindle is outlined by a border of cell inclusions (pl. I, fig. 3 a ; pl. II, fig. 2 a). We can thus see that the spindle elongates during anaphase, as in the chick (Hughes and Swann, 1948). Random linear movement of the chromosomes in metaphase also occurs. This ceases when all move together in anaphase.

Details of individual chromosomes are clearer at this period of mitosis in *Rana* than in *Xenopus*. *Xenopus* has 18 pairs of chromosomes, with no great variation in size among them. The diploid number of *R. arvalis* is 24 and of *R. temporaria* 26 (Wickbom, 1945). In both species there is a comparatively steep gradation in size and length of the chromosomes, and the few large pairs are conspicuous at metaphase (pl. II, fig. 2 a). In the flattened cell in tissue culture these large pairs are extended in the plane of the cover-slip, and their split condition is clearly apparent. The early stages of the separation of the daughter chromosomes in *Rana* are shown in pl. II, fig. 2.

This series of photographs shows that the separation of the chromosomes in anaphase begins at one point, which we can identify as the centromere (pl. II, fig. 2 c–e). As the centromeres move apart we see that their position on the chromosomes is median, for the other arm of the chromosome then comes into view (pl. II, fig. 2 h). The two distal ends of each chromosome pair are still in contact when the centromeres are already some 10 microns apart (pl. II, fig. 2 i : compare text-fig. 3).

The sequence of changes in the daughter chromosome groups from anaphase onwards is similar in both *Xenopus* and *Rana*. During anaphase the chromosomes contract by nearly half their length ; the daughter groups thus become progressively more compact. In telophase the chromosomes appear to coalesce (pl. II, fig. 3 d).

Later, however, the apparently fused mass expands to a network of tangled threads (pl. II, fig. 3 e). At this stage the nuclear membrane appears as an envelope to the network, and gradually becomes more distinct. The network then disintegrates into its constituent chromosomes ; one or two nucleoli appear, and rapidly grow in size (pl. II, fig. 3 f). Probably the reduction of the chromosome segments of this stage to the pro-chromosomes of the mature interphase nucleus is not complete for several hours.

Where the mitochondria in a dividing fibroblast of *Xenopus* are present mainly in the filamentous form, it can be seen that they are comparatively still during mitosis, but resume the active movement of interphase at the moment when the daughter cells become flattened at late telophase.

THE INTERPRETATION OF THE RADIAL STAGE.

The natural way of regarding this stage in mitosis is as a metaphase plate, with the spindle in the clear area in the centre. Later, the spindle may well increase in length, as in the chick (Hughes and Swann, 1948), and be forced out of its first position normal to the plane of the cell by the closeness of the upper and lower cell walls. So the spindle rotates into a position where its

axis corresponds to the long axis of the cell, and as it does so, it either carries the metaphase plate with it, or the chromosomes are re-arranged in the new position, as in *Xenopus*.

If this interpretation is correct, the rotation of the metaphase plate is associated with the flattened form of a fibroblastic cell and would not be expected to occur in more rounded cells. The newt erythroblast (Comandon and Jolly, 1917) passes straight from a radial stage (stade d'étoile mère) into anaphase, without rotation. The direction of anaphase is such that the axis of the spindle in metaphase must lie in the plane of the " figure stellaire."

Again, the arrangement of the chromosomes must be related to the dimensions and form of the cell. The newt erythroblast is ovoid and small; the major axis is only about 22 microns in length. It may be that in such a cell in metaphase the chromosomes can only be packed in a radial form in all three dimensions.

The form and arrangement of the spindle in both the fibroblast and the erythroblast need further study, for which the polarizing microscope is necessary.

TIME RELATIONS.

In Table I are given the data for the time occupied by the various phases of division, measured from our ciné records for the three species which we have studied. The range of values and the corresponding number of examples are there given.

TABLE I.—TIMES OF THE PHASES OF MITOSIS, IN MINUTES FOR EACH SPECIFIED MATERIAL.

	Prophase.	Radial stage to beginning of anaphase.	Anaphase to end of cleavage.	End of cleavage to appearance of nucleoli.
Rana (fibroblast)	>32 (1)	20·7–29 (3)	5·9–10·8 (7)	16·5 (1)
Xenopus (larvæ fibroblast)	—	14·5 (2)	5–14 (12)	3·8–9·5 (7)
Triton				
(liver fibroblast) ..	>18 (1)	16·8–38·3 (3)	14·4–26 (3)	28 (1)
(erythroblast) at 22° C.	—	22 (1)	34–28 (2)	—
(Comandon and Jolly, 1917)	—	—	—	—
(erythroblast) at 25° C.				
(Jolly, 1904)	—	23 (1)	17 (1)	—

Our records begin in either prophase or metaphase. The time occupied by the phase which is thus only partially recorded is indicated by the sign >, indicating that the full period was more than that given.

Our records are deficient with respect to prophase. Only one prophase has been followed with the ×95 objective, namely in the newt, illustrated in pl. III, fig. 4. One prophase in the frog has been followed with the ×40 objective, but the detail seen in this record is not sufficient to warrant much stress being laid on the duration of this prophase.

Table I suggests that from metaphase onwards the time occupied by mitosis

in the amphibian cells bears some relation to the size of the cell. One could estimate that the total time of mitosis is for the frog $1\frac{1}{2}$ hours and for the newt about 2 hours.

In Table I we have also included some further data which relate to mitosis in the newt erythroblasts taken from Jolly (1904) and Comandon and Jolly (1917). These figures have been extracted from the two serial records of division given by these authors in their papers. It is clear that the duration of two given stages of division of erythroblasts at 25° lie within the range of the corresponding times which we have measured.
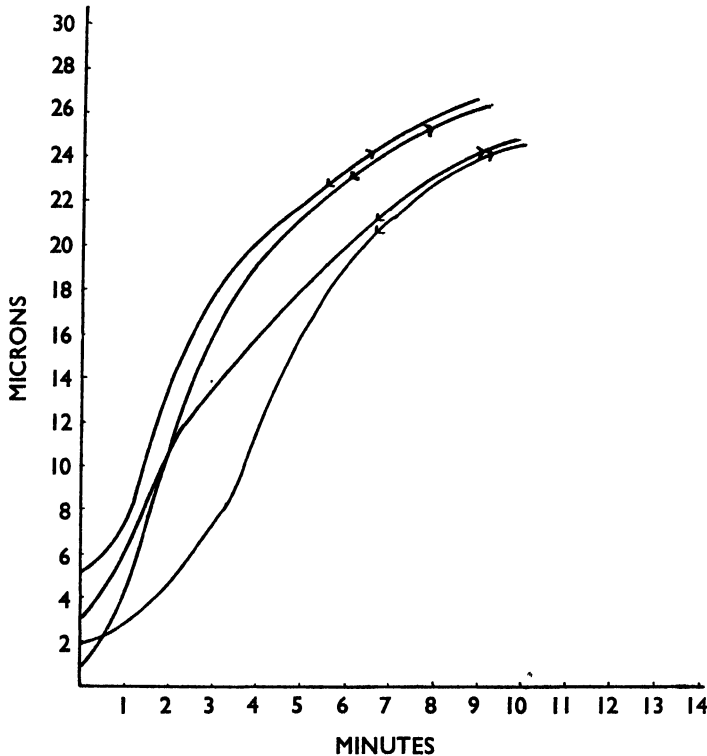
Fig. 1.—*Rana temporaria*, testicular fibroblasts. Group of 4 anaphase curves at 26° C., measured from axial centromeres. The beginning and end of cell cleavage are marked on each curve.

RATE OF ANAPHASE MOVEMENT.

The distance between the groups of daughter chromosomes during anaphase was measured in our ciné records, and these distances were plotted against time. The curves thus obtained are given in text-figs. 1–5. In text-fig. 4 are shown the average curves for *Rana*, *Xenopus*, and *Triton*, together with that for the chick, the anaphase movement of which was studied in a previous paper (Hughes and Swann, 1948).

In the cells of the chick the daughter chromosomes form compact groups, due to their small size ; the distances plotted are mean values between points in the centre of each group. In amphibian cells in anaphase the distance which can most accurately be measured is that between centromeres, for they all lie

on a regular surface. However, owing to the concavity of this surface in late anaphase the centromeres in or near the axis of the spindle travel a shorter distance than the more peripheral ones. Again, the ends of the longer chromosomes travel a much shorter distance (text-fig. 3). Consequently, in comparing anaphase movement in different species one of these three possible curves must be chosen as a standard ; we have used that for the distance between the axial centromeres. The error in comparing this with the mean distance curve in the chick is relatively small.
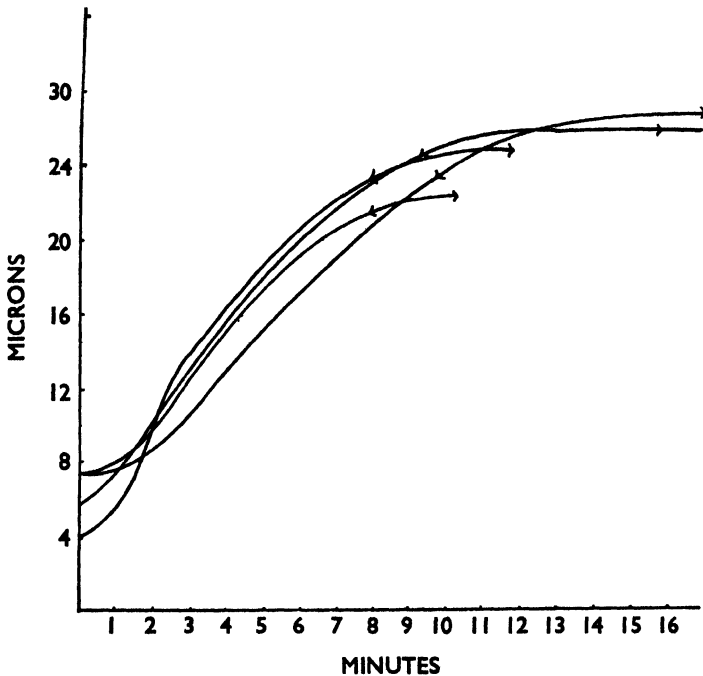
Fig. 2.—*Xenopus lævis,* larval fibroblasts. Group of 4 anaphase curves at 26° C., measured from axial centromeres. The beginning and end of cell cleavage are marked on each curve.

The amphibian anaphase curves are sigmoid in form. The inflection in early anaphase is clearly marked, although variable in extent. In the chick (Hughes and Swann, 1948) the anaphase curve rises steeply from the origin, and the maximum velocity is attained as soon as the movement of the chromosomes can be detected. This extreme reduction of the initial inflection of the chick anaphase curve may be associated with the small size of the chick chromosomes.

In text-fig. 5 the velocity of the chromosomes in each species has been plotted against displacement, and the similar curve for the chick has been included from Hughes and Swann (1948). These curves begin only at the point where the velocities of the chromosomes can be measured with any approach to accuracy, and so omit the rise from the origin which is a consequence of the initial sigmoid inflection. The velocity distance curves for *Xenopus* and *Rana* are taken from the average anaphase curves of text-fig. 4, but that for *Triton* is based on a single record of anaphase.

2

3

4
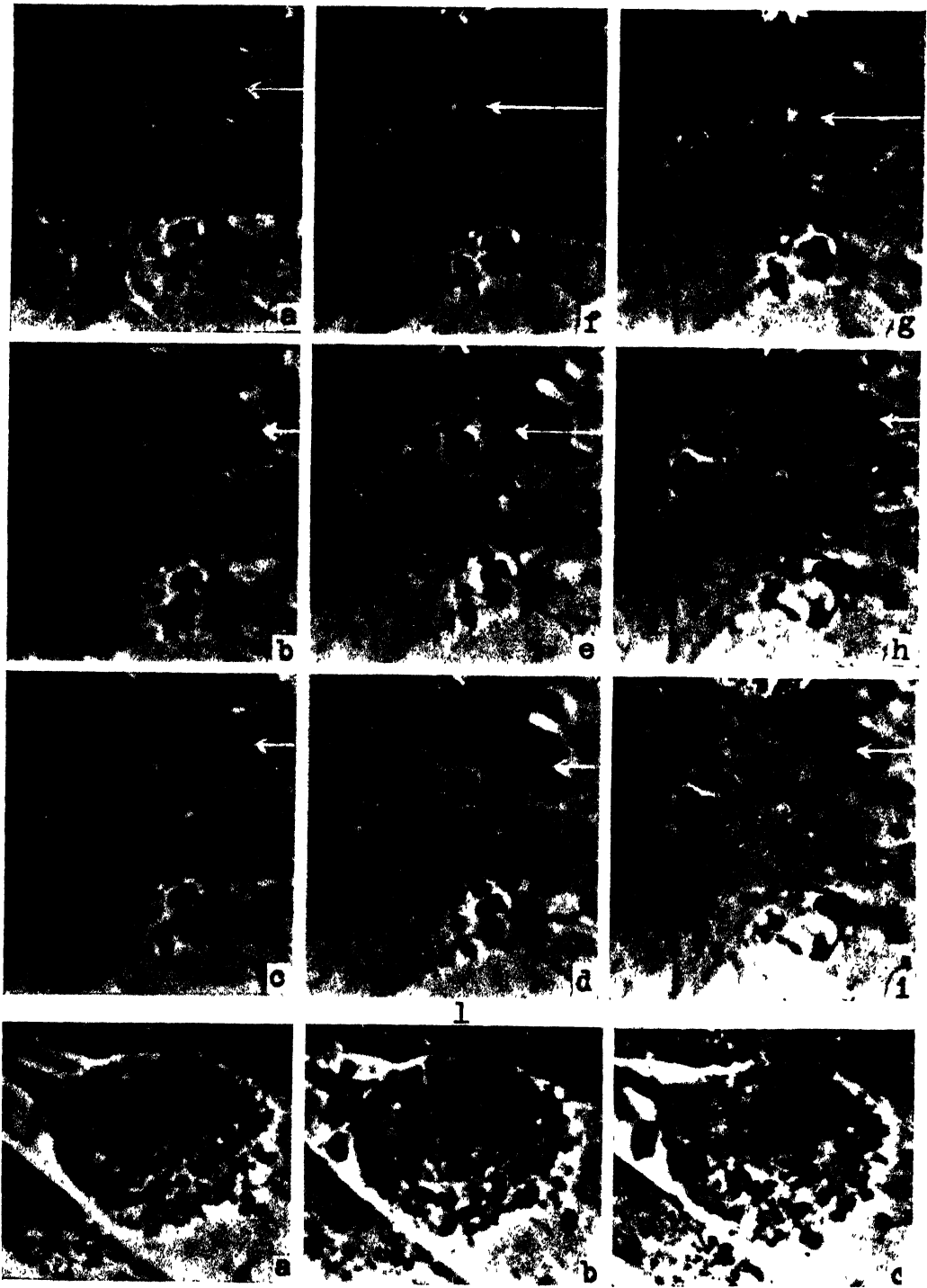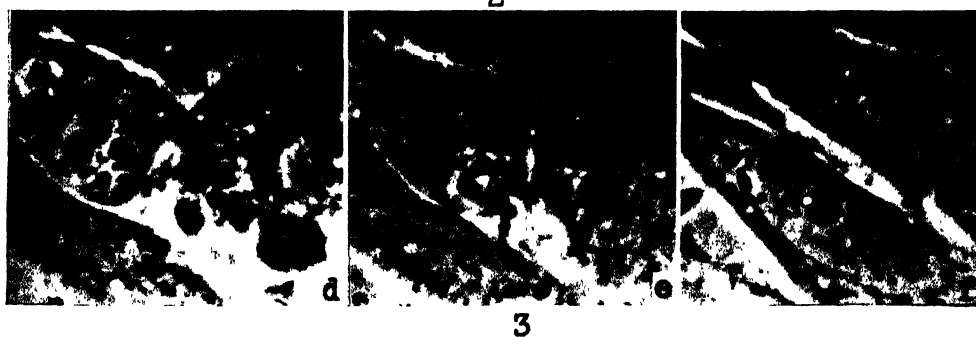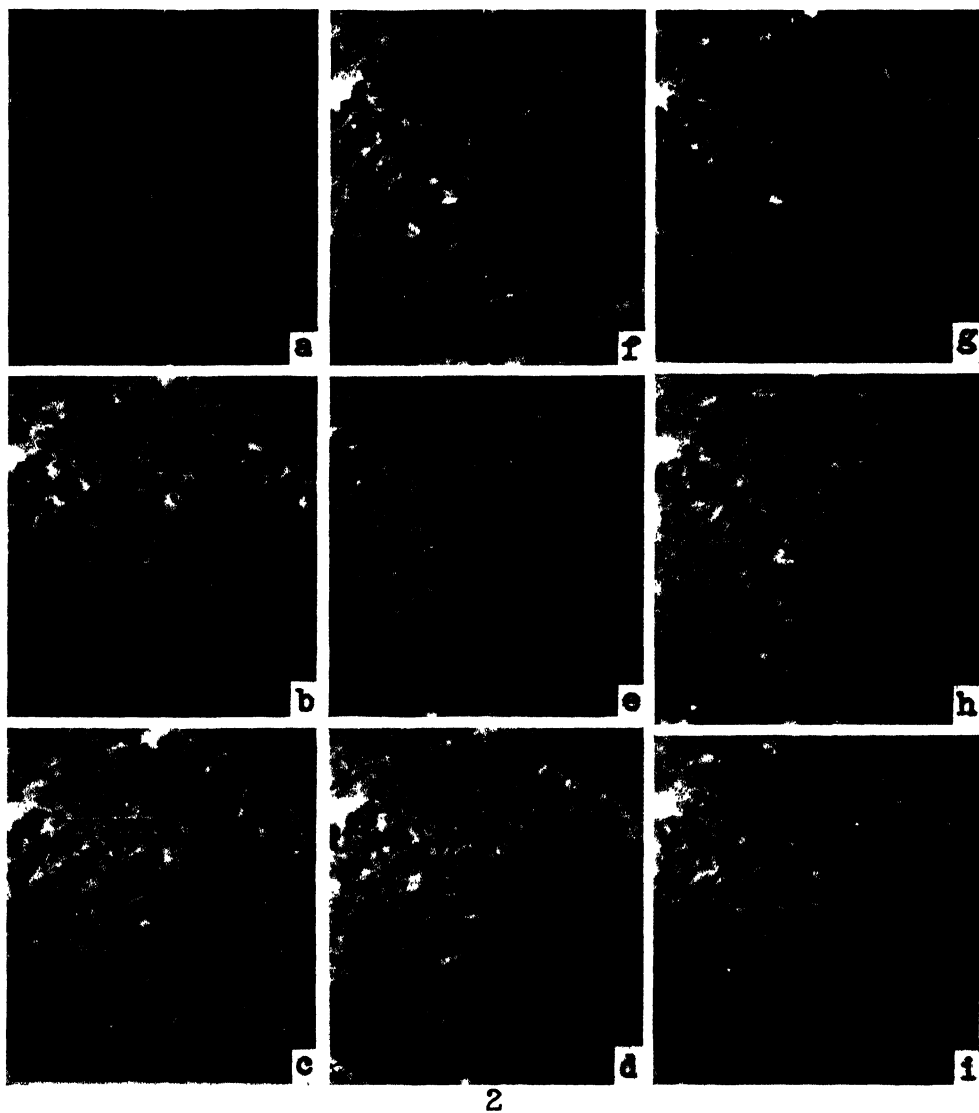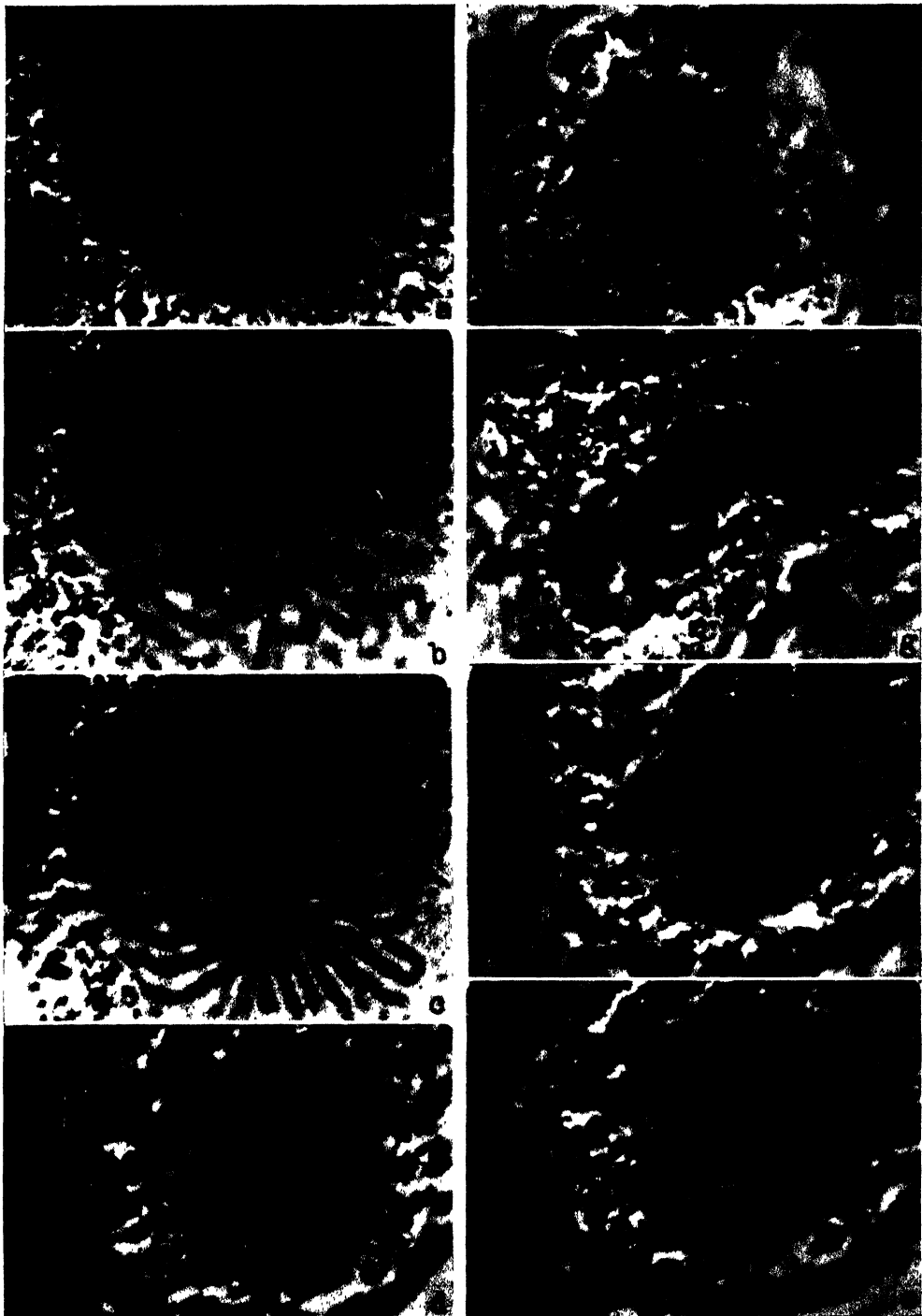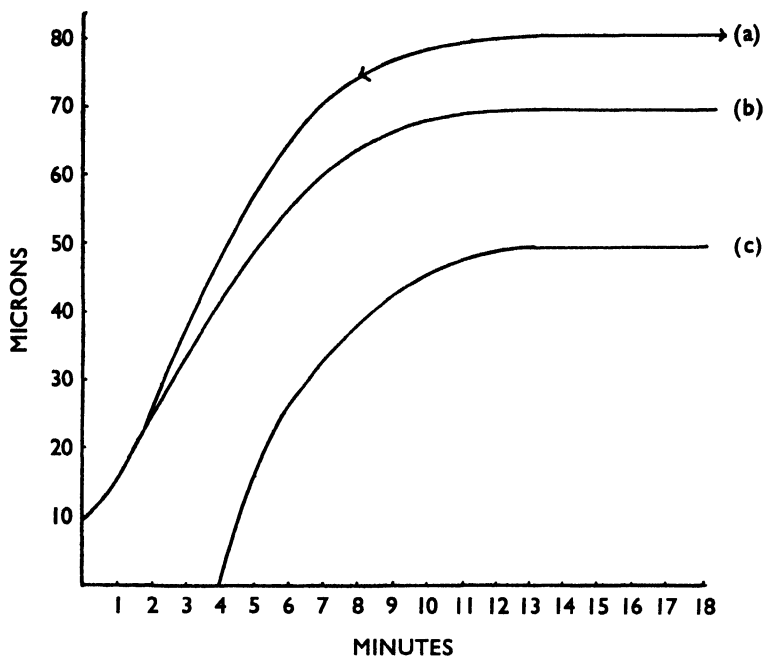
Fig. 3.—*Triton cristata*, liver fibroblast. 26° C. Anaphase curves, plotted from (*a*) peripheral centromeres, (*b*) axial centromeres, (*c*) inner ends of the chromosomes. On (*a*) is marked the beginning and end of cleavage.
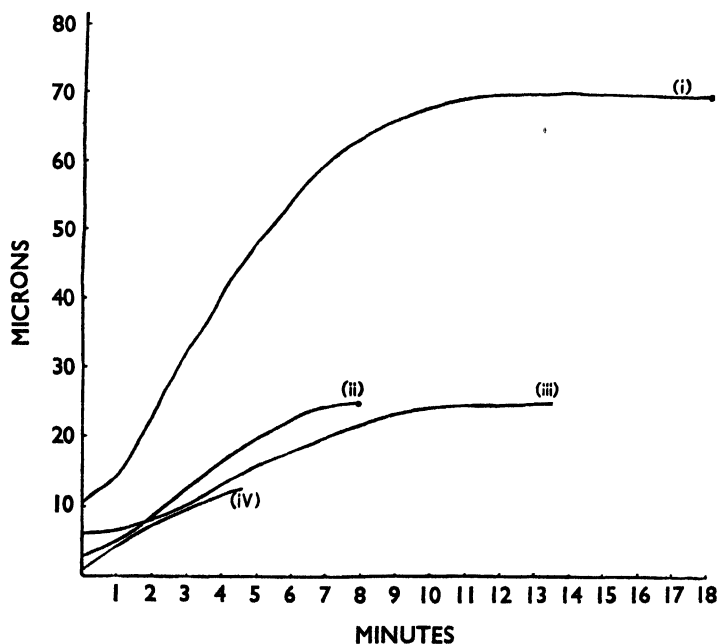
Fig. 4.—Anaphase curves for axial centromeres of (i) *Triton*, (ii) *Rana* (averaged from Fig. 1), (iii) *Xenopus* (averaged from Fig. 2), (iv) Chick average curve, for comparison.

The average curves of text-fig. 5 indicate that the maximum velocity of a chromosome in *Xenopus* and *Rana* is about 2 microns per minute. Measurement of a single pair of centromeres in *Rana* gave a value of 2·4 microns per minute. In *Triton* the maximum velocity is large and in the single instance here plotted exceeds that of the chick. From the point of maximum velocity there is a steady negative acceleration with further displacement.
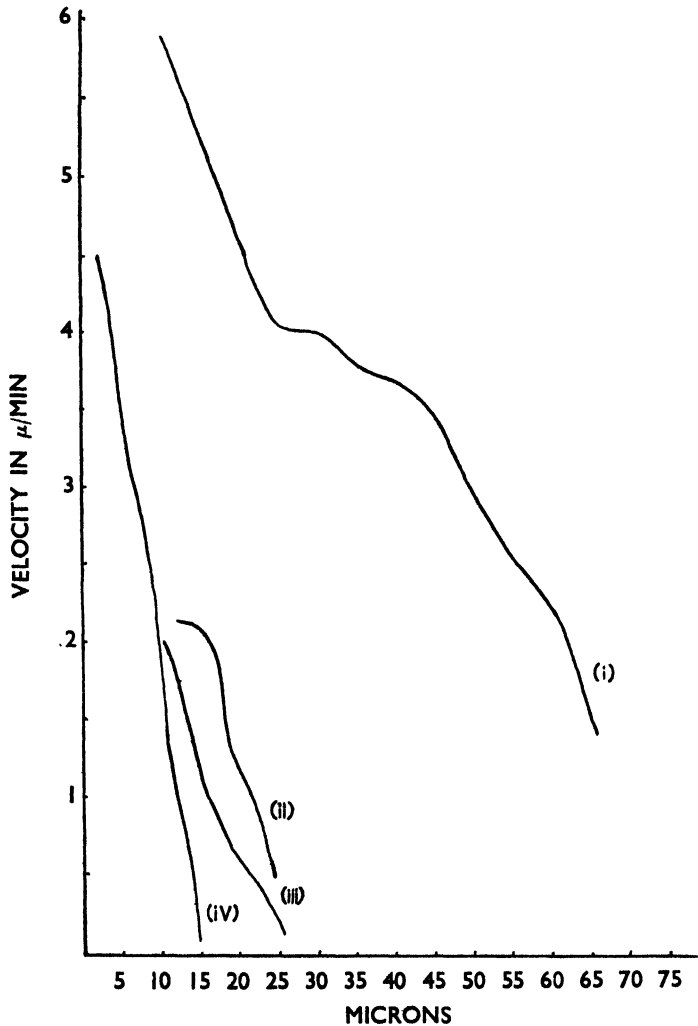
Fig. 5.— Velocity distance curves of anaphase movement, obtained from the data of Fig. 4. The rates of movement refer to single centromeres.   (i) *Triton*, (ii) *Rana*, (iii) *Xenopus*, (iv) *Gallus*.

In the newt it is possible to compare the anaphase of two different types of cell, namely the liver fibroblast in tissue culture, and the erythroblast studied by Comandon and Jolly (1917). The time occupied in the anaphase and cleavage of both cells is roughly the same, as we have noticed above. Yet the erythroblast is smaller, and in it the distance travelled by the chromosomes is about one-third of that in the fibroblast. Comandon and Jolly estimate the velocity of the daughter chromosomes in anaphase to be not more than 1·5 microns per minute. In the fibroblast the initial velocity is 3–4 times this figure.

SUMMARY.

1. Cells in cultures of amphibian tissues were studied by ciné-photomicrography, by phase-contrast technique.

2. The changes in shape and type of movement of the mitochondria in fibroblasts of *Xenopus lœvis* are described.

3. Observations on cell division in *Triton*, *Rana*, and *Xenopus* are described.

4. The time occupied by the phases of division and the rates of anaphase movement are compared for the three species.

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DESCRIPTION OF PLATES.

Plate I, fig. 1 *a–i*.

Part of an intermitotic fibroblast of *Xenopus lœvis* in tissue culture, photographed at intervals to show mitochondrial movement, in particular of the mitochondrium indicated. × 1200.

b 63 seconds after *a*	*e* 60 seconds after *d*	*h* 350 seconds after *g*
c 38 ,, ,, *b*	*f* 106 ,, ,, *e*	*i* 6 ,, ,, *h*
d 120 ,, ,, *c*	*g* 31 ,, ,, *f*	

Plate II, fig. 2 *a–i*.

A fibroblast of the frog in tissue culture, during division, showing the first 45 seconds of anaphase, particularly to illustrate the separation of chromosomes. × 1200 ; interval between successive pictures, 5 seconds, except that between *h* and *i*, which is 10 seconds.

Plates I and II, fig. 3 *a–f*.

A fibroblast of *Xenopus lœvis* in tissue culture, during division, to illustrate the progress of mitosis from metaphase onwards. × 1000. The interval of time between the first and successive exposures is as follows :

b 7·6 minutes after *a*	*d* 15·0 minutes after *a*	*f* 55 minutes after *a*
c 10·0 ,, ,, *a*	*e* 20·8 ,, ,, *a*	

Plate III, fig. 4 *a–h*.

A newt fibroblast in tissue culture during mitosis from prophase to telophase. × 1200. The intervals between the first and the successive exposures are as follows :

b 5¼ minutes after *a*	*e* 44½ minutes after *a*	*h* 76 minutes after *a*
c 8¼ ,, ,, *a*	*f* 48 ,, ,, *a*	
d 43 ,, ,, *a*	*g* 61 ,, ,, *a*	

535.41 XIII.—THE USE OF INTERFERENCE FILMS IN OPTICAL
INSTRUMENTS.

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Introduction.

This paper deals with some of the ways in which the phenomena of interference can be made to produce effects useful in optical instruments. These interference effects are induced by applying thin transparent films of suitably chosen material to the surfaces of lenses, prisms, and optical windows.

A common application of interference already well known is the antireflection film, known as surface-coating or " blooming," now very widely used for improving the image in binoculars, camera lenses, and many types of military instruments.

A remarkable contrast to the anti-reflection film is the high-reflection film, remarkable because its reflection factor of 30 p.c. in white light is achieved with a single film of exactly the same optical thickness as that of the anti-reflection film. By a suitable combination of films of these two types built up in alternate layers, the reflectivity for a particular colour can be still further increased ; the transmitted beam becomes correspondingly coloured in the complementary colour.

With the addition of metallic reflecting layers on each side of an interference film, a filter can be constructed which transmits almost monochromatic light. This is the interference filter, one of the latest and most striking applications of the principle of interference.

All these devices are simple variations on the same theme, interference due to a thin, parallel film. The film is a means of readjusting the distribution of energy between the beams reflected and transmitted by a glass surface. Its action is, broadly speaking, to induce multiple reflections within the film and to adjust their phase relationships so that when they are combined in the emergent beams the intensities of reflection and transmission are decreased or increased as required.

The Anti-reflection Film.

The theory of thin films has been dealt with in many previous papers (Blodgett, 1939 ; Greenland, 1943 ; Bateson, 1947) ; indeed, the basic principle of these modern devices has been displayed in the standard text-books for very

many years. It was, apparently, a failure to appreciate their practical possibilities together with a lack of the means of producing the films which postponed their application for so long.

The ray reflected from any point on the surface of a film is a composite ray, made up of the reflected component of the incident ray at that point and of the contributions from other incident rays which emerge at that point after multiple reflections within the film. Adding all these components together, with due regard for their phase relationships (which are determined by the film thickness t and refractive index μ, wavelength of light λ and angle of reflection θ), calculation shows that for a film of refractive index lower than that of its glass support the reflected ray is reduced to a minimum intensity when

$$\mu t \cos \theta = \lambda/4 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

and that the intensity of reflection is zero when the refractive index of the film is equal to the square root of that of the glass. The quantity μt is the optical thickness of the film, so that for normal incidence ($\theta = 0°$) the optical thickness of the film must be equal to $\lambda/4$; hence such a film is sometimes known as a quarter-wave film.

Anti-reflection films are usually required to be effective in approximately white light, whereas equation (1) can only be satisfied for a pure colour. It happens, however, that if the wavelength is increased or decreased from the value required to satisfy the equation, the intensity of reflection only increases slightly within the range of wavelength included in the visible spectrum. If, therefore, the optical thickness is chosen so that the wavelength of minimum reflection is near the middle of the visible spectrum (the yellow-green), the reflectivity for the colours red and violet is not much higher and the film has its characteristic dull purple appearance.

According to equation (1) the angle of incidence is also a variable to be taken into account. Here, again, however, the value is not critical near normal incidence, a variation of 20° from the normal having very little effect in increasing reflectivity.

The material of which the films are made must, of course, be durable, mechanically and chemically, as well as optically suitable. This usually means that its refractive index is rather higher than the optimum value, but the hardness and durability of the film offset the slight loss of optical efficiency (magnesium fluoride is a material commonly used and its refractive index is 1·36 for a thin film).

It is apparent, then, that although equation (1) and the " square root " condition for the refractive index of the film must be satisfied for a complete absence of reflection, a very satisfactory practical compromise can be achieved with a durable film in white light at angles of incidence up to 20°.

Reduction of the light intensity in a particular direction is sometimes called " destructive " interference. This is a misleading term, since, as already explained, interference only results in a redirection of energy and no energy is lost. This absence of any absorption is an important feature of all types of interference film. In the case of the anti-reflection film, the light energy is transferred from the reflected to the transmitted beam, so that the intensity of

the transmitted beam is increased in exact correspondence with the decrease in intensity of the reflected beam.   This may be verified by a calculation similar to that made for the reflected ray, the only difference being in the phase relationships of the components of the rays (the components of the transmitted rays are exactly in phase when those of the reflected rays are completely out of phase).

THE HIGH-REFLECTION FILM.

Suppose now that the refractive index of a quarter-wave film is changed to a value higher than that of the glass support.   All the reflections at the film-glass boundary which in the low-refractive-index film were in the lighter medium now find themselves in the denser medium.   The effect is to interchange the phase relationships of the components of the reflected and transmitted rays, so that the quarter-wave film now gives maximum reflection and minimum transmission.   Moreover, the intensity of this maximum reflection depends on the refractive index of the film (and of the glass support), so that it is only limited by the materials available.   In fact, a reflectivity for white light of 30 p.c. is attainable with a quarter-wave film of zinc sulphide.   This gives a remarkably metallic appearance to the film and it is difficult to realize that the material of which the film is made is really transparent.

The most important feature of these high-reflection films is that, being of truly transparent material, there is no absorption in the film, so that with a reflectivity of 30 p.c. the transmissivity is 70 p.c.   A metallic film having a reflectivity of 30 p.c. would have a transmissivity of only 25–30 p.c.   Since the dependence of transmission and reflection factors on wavelength and angle of incidence is similar to that of the anti-reflection film, the high-reflection film gives a " white " reflection and an almost neutral transmission and so may be used as a neutral beam-splitter (Greenland, 1946 ; Banning, 1947).

USES OF SIMPLE INTERFERENCE FILMS.

The anti-reflection and high-reflection films are single films of well-defined thickness, and given the necessary apparatus are comparatively easy to make. This has made possible their application on a commercial scale in modern optical instruments.   Probably the most useful function of the anti-reflection film, at least as far as non-military instruments are concerned, is the reduction of glare due to reflections from the surfaces of lenses and prisms.   An excellent example of an instrument which benefits greatly from the surface-coating of its lenses is the metallurgical microscope (Taylor, 1945).   The illuminating beam passing down through the objective sends back reflections from both surfaces of each component, so spreading a haze of light over the image ; it is now the practice of some makers to " bloom " all metallurgical objectives.

The simple high-reflection film is of more limited application than the anti-reflection film, but its ability to reflect and transmit efficiently without colouring either beam makes it peculiarly suitable for application to the vertical illuminator of the microscope already mentioned (Taylor, 1945).   It reflects 30 p.c. of the

illuminating beam down through the objective instead of the 8 p.c. reflected by the plain glass reflector. Taking into account the transmission of the reflector (70 p.c. coated, 92 p.c. uncoated) the net result is that with a coated reflector the equivalent of about 20 p.c. of the light from the lamp is available for illuminating the object, whereas an uncoated reflector gives only 7 p.c.

The Interference Filter.

The intensity of the light passing through an interference film depends, as we have seen, on the effect of the addition of components to the directly transmitted ray by means of multiple reflections between the boundaries of the film. Now, if the reflectivity of the boundaries of the film is made very high, these added components have a much greater influence on the intensity of the transmitted beam, and, in particular, the intensity depends to a very great extent on the phase relationships of all the components. In fact, if the reflectivity can be raised to, say, 85 p.c. at each boundary, the amount of light transmitted by the film will be negligible except when the large number of multiple reflections are exactly in phase with the direct ray. This means that if the thickness of the film and the angle of incidence are constant, only light of certain very narrow wave-bands will be transmitted by the film. The film is then a very efficient colour filter. A filter of this kind is called an interference filter. It is made by coating glass with a " sandwich " of two semi-transparent metal films separated by a transparent film of dielectric such as magnesium fluoride. The width of a transmission band is of the order of 100 Å.U. and the amount of light transmitted at the " peak " wavelength may be as much as 30 p.c. By careful control of the thickness of the dielectric film the mean wavelength of a transmission band can be adjusted to within about 10 Å.U. There may be one or several transmission bands within the visible spectrum, depending on the desired width of the bands. Such a filter is easily capable of isolating any one of the lines of the low-pressure mercury-discharge spectrum. Those interested in the theory of the interference filter will recognize that it is the same in principle as that of the Fabry-Perot Etalon.

Although interference filters are more complicated in construction and require more accurate control in their manufacture than the anti-reflection film, the difficulties of commercial production are economical rather than physical. As far as the technical side is concerned, there is no reason why they should not shortly become generally available in this country.

Multiple-film Colour Filters.

The simple high-reflection film is, of course, slightly sensitive to wavelength, though the difference in reflectivity of different colours is hardly noticeable to the eye. Its reflectivity can be increased from 30 p.c. to about 35 p.c. if a quarter-wave film of magnesium fluoride is put on to the glass before the quarter-wave zinc sulphide film is deposited, but as the interference effect of two films is now involved, the dependence of reflectivity on colour begins to be noticeable, the reflection being faintly greenish and the transmission slightly purple. By

piling alternate films of high and low refractive index one on top of the other and making the optical thickness of each the same (e.g. one quarter-wavelength of green light) the reflectivity for the chosen wavelength may be increased until it approaches 100 p.c. In practice, a limit is set by the tendency of a thick pile of films to recrystallize, but a reflectivity of 60 p.c. has been reached without much difficulty (Greenland, 1946). At these higher reflectivities the effect of wavelength is very marked, so that both the reflected and transmitted beams are more strongly coloured. An interesting property of such a filter is that when it is illuminated with white light the colours of the reflected and transmitted beams are truly complementary. They can, in fact, be re-combined to give white light. Films of this kind are beginning to be used in place of absorption filters for splitting white light into two or more coloured components.

Zonal Retardation Plates for Phase-contrast Microscopy.

A reference to the retardation plates used in phase-contrast microscopy may not altogether be out of place, because although an interference film, as such, is not required, the phase retardation is usually brought about by the use of a film of the same composition and order of thickness as the anti-reflection film.

The details of a microscopic object in which there is very little contrast due to differences in optical density can be made much more visible by emphasizing any differences of refractive index. The emphasis is achieved either by retarding the rays diffracted by the object so that they lag behind the directly transmitted rays by one quarter-wavelength (positive contrast) or by retarding the direct rays by the same amount (negative contrast). By so doing, the distribution of light in the image formed by the objective is made to depend on differences in the retardation of rays passing through the object instead of on differences in the absorption of these rays. The diffracted or direct rays are retarded by putting a glass disc, having a film of transparent material over part of its surface, at the back focal plane of the objective, where the diffracted rays and undiffracted rays are separated physically. The configuration of the coated area depends on the shape of the condenser stop (which is usually an annulus) and also on whether positive or negative contrast is required. The retardation effect of a film (thickness t, refractive index μ) for light of wavelength λ is $(\mu - 1)t/\lambda$ wavelengths, and since this retardation is to be one quarter-wavelength the thickness t must be $\lambda/4(\mu - 1)$. For magnesium fluoride this works out to be very nearly three times the thickness of an anti-reflection film. A more detailed description of the theory of phase contrast and its practical application is given in a paper by Taylor (1946).

The Technique of Film Deposition.

Volatilization of the film materials by heating in a high vacuum is the almost universal method of depositing interference films of all the kinds described. This statement must be qualified by saying that two chemical methods are also being used, but in this country only on a small scale.

The space in which the volatilization is carried out is enclosed by a bell-jar of glass or steel about 18 inches in diameter and 24 inches in height, resting on a flat base-plate. This space is evacuated by a diffusion pump assisted by a mechanical pump to a pressure of less that 10^{-4} mm. of mercury. In a vacuum of this kind metals such as aluminium and silver and minerals such as magnesium fluoride and zinc sulphide will evaporate readily when heated, and will condense in a smooth film on any unheated surface *facing* the heater from which the material is evaporating (the distribution of the material depends only on distance from heater and shape of heater—there is no diffusion of vapour, as at higher pressures). The metal or mineral is usually heated by placing it on a broad strip of molybdenum or tungsten foil through which an electric current may be passed.

The interference films depend for their effectiveness on a close adjustment of their thickness. It is fortunate that they proclaim their thickness with sufficient accuracy by the colour which they reflect when illuminated with white light, so that it is usually sufficient to watch the reflected image of a white lamp while deposition proceeds and to switch off the evaporator heater current when the appropriate colour is reached. In the special case of the interference filter it is necessary to use a more accurate method, in which the light reflected from the growing film is analysed by means of a spectroscope.

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576.85 XIV.—FLAGELLA AND MOTILITY OF *VIBRIO METSCHNIKOVII*

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ONE PLATE

Vibrio metschnikovii is generally believed to be driven along by a single terminal flagellum, occasionally by two. From one electron photograph van Iterson (1947*a*) " got the impression " that such a single flagellum originated from a basal granule ; in a further publication (1947*b*) this same flagellum is stated by her to originate from a definite blepharoblast. Mudd, Polevitzky, and Anderson (1942) suggested continuity between protoplasm and wavy threads lying round *Vibrio schuylkiliensis* (probably a synonym of *V. metschnikovii*) in an electron photograph, but saw no basal granule.

One of us has advocated a theory that motile bacteria are not moved by flagella, but by gyrating, undulating movements of the bacterial body, which is really not rod- but spiral-shaped, and that the so-called flagella are passive wavy threads derived from the mucous coat, which become twisted into a tail during active movement (Pijper 1946, 1947*a*, 1947*b*, 1947*c*, 1948*a*, 1948*b*, 1949*b*). The theory does not apply to the very large spirillums, such as *Spirillum volutans*, where the so-called flagella are a continuation of the cell wall proper (Pijper 1949*a*). These revolutionary views were derived from studies with the sunlight darkground microscope previously described (1940, 1941*a*, 1941*b*, 1942). Vibrios seemed indicated as the next object of study.

OBSERVATIONS WITH SUNLIGHT DARKGROUND MICROSCOPY.

All observations were made on overnight broth cultures kept at 21° of a strain of *V. metschnikovii* received from the National Collection of Type Cultures of the Lister Institute.

This vibrio showed very surprising features. Motility was very good and the vibrios moved as was expected with a gyrating, undulating movement, very similar to that of typhoid bacilli (1946, 1947*a*). A surprise was that instead of showing an appendage or flagellum at the rear end, most of them seemed to carry a rod at the front end. This gave them the appearance of ships with a bowsprit or a microbe with a snout. During fast movement this snout seemed straight and appeared attached to the pole, extending in the direction in which the vibrio was going. Figs. 1–12 are copies from consecutive pictures of a
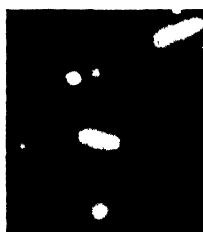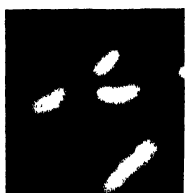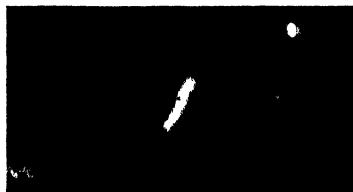
16-mm. cinemicrographic film. They demonstrate a small, slightly curved vibrio moving in the direction of its snout.

On closer inspection this snout was seen to be not a straight rod but a fine coil, and there are slight indications of this in figs. 1–12. On slowing down the coil became quite obvious, as in fig. 13, which shows a vibrio which is moving slowly in the direction of its snout.

Many vibrios were followed through a number of fields, their bodies exhibiting gyrating, undulating movement with the coil-shaped snout in front all the time, and pointing in the direction of movement. Others, however, showed fast locomotion with the snout not straight in front, but bent at an angle, the snout through the gyrating movement of the body describing the surface of a cone, as in figs. 14 and 15, which show vibrios at high speed. Figs. 16 and 17 show the same phenomenon, but here speed was slower and the finely coiled structure is evident. In fig. 17 the snout is moving at nearly a right angle to the body. The attachment of the snout is on the whole polar, but not always exactly át the pole, as instanced in fig. 18.

Occasionally the fine coil of the snout was seen to split into two, sometimes three finer coils. These then described conal surfaces of their own, often of different size, and sometimes they came together again and formed one snout. In cases where one of the split-off coils had been shorter than the other, this then remained visible in that the new combined snout was proximally thicker than at its end. These happenings did not necessarily interfere with speed of swimming.

These observations are rather against the conception that the vibrio is driven along by a terminal flagellum. Further observations brought further surprises. " Reversal " of movement, meaning that a vibrio suddenly changes its direction of movement and goes back to where it came from, occurred very frequently. The snout then became a tail ; and when another reversal followed, the tail again became a snout. This is quite different from what happens with *Salmonella typhi*, where on sudden reversal the tail floats to the other end and there again appears as a tail (1946, 1947a). Whenever through reversal a snout became a tail, it appeared to be longer and thinner and therefore less easily visible. This is the reason why the impression was gained at first that there was a majority of snouts. Occasionally vibrios were found in possession of both a snout and a tail, and here too the snout always was thicker and shorter and more easily visible. When such vibrios stopped movement or moved slower, both snout and tail readily showed their coil-like structure. Fast-swimming vibrios often seemed to have neither snout nor tail, but when such an individual performed a reversal, it happened that a snout suddenly became visible. A further reversal made the snout disappear again, or left it just faintly visible as a tail. It also happened that a vibrio seemed to have a snout only, but on reversal the snout became a tail and a new snout appeared at the frontal end.

The explanation of these strange happenings is quite simple. *V. metschniko-vii* obviously possesses polar appendages, sometimes only one, at one pole, sometimes one at each pole. These appendages have the shape of a fine coil, as in fig. 13. When such a vibrio moves fast in the direction of the appendage, the

coil becomes somewhat compressed and slightly broader, and can give the impression of a straight and fairly heavy rod, as in figs. 1–12. When the vibrio reverses, the appendage naturally becomes drawn out, and thus longer and thinner, and may become too thin to be easily visible.

Collisions between vibrios occurred frequently, much more often than with *Salmonella typhi* (1938, 1941*b*, 1946). If one bumped its snout, it reversed and swam away quickly, and then reversed again and went on in the original direction.

Collisions often lead to entanglements of snouts, especially when movement was rather slow. The clumps arising from this often consisted of three individuals, and the clumps kept up a rotating movement of the whole clump. At other times the clump remained more or less on the same spot and then the bodies often kept up ceaseless undulating, gyrating movements whilst the twisted combined appendages remained curiously quiet. Sometimes one of the bodies of a clump kept on moving while the rest of the combination kept still. It also happened that the tail of one vibrio got entangled with the snout of another one and that the complex kept on moving in the direction of the first one, the gap between the two, and therewith the entangled snout and tail, becoming lengthened and drawn out more and more during the forward movement. This is not easily compatible with the idea that the appendages provide the moving force.

Observations with Pointolite Darkground Microscopy.

In previous work (1947*a*) it had been found that the appendages of typhoid and proteus bacteria increased in thickness and visibility on suspending or growing the bacteria in colloid solutions of various kinds. The thickening proved to be due to the colloid substances precipitating in granular fashion on to the appendages, providing them ultimately with a continuous sheath. The appendages in their thickened state then became visible with simple darkground methods, without the use of sunlight (1947*a*). It seemed appropriate to try such procedures on the appendages of *V. metschnikovii* in the hope of making them also visible with simpler darkground methods than sunlight microscopy. The following substances were tried in solutions of varying strength : methyl-cellulose, gum arabic, gum acacia, gum indican, mucin, starch, glycogen, gelatine, agar agar. No thickening, however, was observed, except occasionally and slightly with methylcellulose. It was revealed, however, that even in ordinary broth without colloid additions the appendages of *V. metschnikovii* as described above for sunlight darkground microscopy became reasonably well visible with a Pointolite lamp and a Spencer or Zeiss (cardioid) darkground condenser.

Their visibility was never as good as with sunlight and finer details often remained hidden, but practically all the phenomena mentioned above could be seen with the simpler equipment. Obviously the consistence and refraction of the appendages of *V. metschnikovii* are different from those of typhoid bacilli which remain practically invisible with such simple methods. It is strange that these snouts and tails of *V. metschnikovii* have apparently never been noticed before.

Suspending *V. metschnikovii* in methylcellulose solutions occasionally gave rise to slight thickening of the appendages, and even then this only became apparent after the vibrios had been in the solution for several hours. Motility by that time had ceased and the vibrios probably were dead. The pictures obtained were like figs. 19 and 20. It will be noted that the coils are much broader than in the normal state and that their attachment has shifted to the side of the pole. The similarity of these pictures of dead vibrios to the traditional photomicrographs of flagella-stained material and the electron microscope pictures of van Iterson (1947a) is very striking. A similar attachment, some distance away from the pole, is also visible in some of the other pictures. When a snout splits the attachment of the resulting coils also remains more or less polar ; they never were seen to spread themselves all over the body, as is the case with *Salm. typhi* (Pijper 1946). The attachment is not very strong ; in every preparation loose coils were found floating around. On the other hand, prolonged shaking of cultures failed to break them off. It has been shown that the appendages of *Salm. typhi* disappear after 15 minutes' shaking of the culture (1949b) without motility being affected.

DISCUSSION.

The nature of the appendages of *V. metschnikovii* appears to be similar to but not identical with that of the appendages of *Salm. typhi*. In *Salm. typhi* they are inert, long, wavy threads derived from the mucous covering through the gyrating, undulating movement of the body, and they form a long tail during fast movement. The mucous covering here is of soft consistency and this explains how the bacterium can turn a semi-somersault in its own coat without disturbing the tail, and also that during a sudden reversal the tail drifts to the other end of the body and there becomes a tail again (1946, 1947a). In *V. metschnikovii* the mucous covering apparently is of a denser and stiffer nature, and once it has been twisted into the fine coils described above it remains more or less *in situ*. Its stiffness makes it possible for the vibrio to travel with a snout, and its greater density probably explains its ready visibility with simple darkground methods. On one occasion it proved possible to harden the mucous covering of *Salm. typhi* sufficiently to keep the tail in place after reversal and make it appear as a snout. This was done by growing the bacterium in rather acid broth and suddenly adding a drop of sodium bicarbonate solution to the preparation under the microscope. The sudden change in *pH* value brought about a rapid increase in motility, and many individuals were seen swimming with their tails in front.

SUMMARY.

The supposed flagella of *V. metschnikovii* can be made visible not only with *sunlight* darkground microscopy but also with a Pointolite lamp and a Spencer or Zeiss darkground condenser. The observations thus made lend support to the theory proposed by one of us (P.) that bacterial " flagella " are not motor organs but passive structures derived from the mucous coat.

ACKNOWLEDGEMENTS.

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DESCRIPTION OF PLATE.

PLATE I.

Figs. 1–12.—Enlarged copies from consecutive pictures of cinemicrographic film of *V. metschnikovii*, swimming fast in broth, demonstrating that it moves in the direction of the snout. × 3000.

Fig. 13.—*V. metschnikovii*, in broth, slowly moving in direction of the fine coil which forms its snout. × 3000.

Figs. 14, 15.—*V. metschnikovii*, in broth, moving in direction of snout, which stands at an angle and during movement describes the surface of a cone. Snout really is fine coil, but speed of movement has blurred it into a rod. × 3000.

Figs. 16, 17.—See text.

Fig. 18.—*V. metschnikovii*, in broth, swimming fast in direction of snout, which during movement describes the surface of a cone, and seems to be attached some distance from pole. × 3000.

Figs. 19, 20.—*V. metschnikovii* suspended in methylcellulose solution. Appendage is broader and thicker than normal and appears to be attached some distance from pole. × 3000.

XV.—CONSIDERATIONS AFFECTING AXIAL ILLUMINATION. 535.824.3

By W. G. HARTLEY.

A RECENT communication by Oettlé (1947) draws attention to the necessity for centring the illuminant to the microscope axis.

The method adopted by the present writer, and supposed by him to be general, is to centre the light source in the field of view by manipulating the mirror after the condenser is centred and focused. In effect the light is viewed through a microtelescope and adjusted to it.

Should the path of the light through the condenser be wildly oblique, difficulty may be experienced in focusing the aerial image of the diaphragm in the traditional manner, and this image may be laterally displaced, in which case its shape or colouring becomes asymmetric and centration impossible. To avoid this, the objective and condenser may be focused without previous centration as soon as the field is illuminated, the eyepiece then removed and the condenser centred by inspection of the iris through the objective. The mirror can be adjusted concurrently, and exact centration of the light source, if this is of a nature which permits it, completed when the eyepiece is replaced.

In this connection it may be suggested that the petrologist has a great advantage in the ability to slide in a Bertrand lens for inspecting the back of the objective, and can check condenser centration and aperture whilst the biologist is still removing his eyepiece. The use of the phase-contrast technique may result in the introduction of this useful auxiliary to a new audience.

Furthermore, it is noteworthy that it is usually necessary to take the centration of the iris to the condenser on trust.

The problems of illuminant centration are greatly aggravated when a lamp condenser is used, as in this circumstance there are two separate optic axes to be aligned, instead of a single point to be made axial. In addition, it is not infrequently the case that the centres of rotation of the lamp during elevation and training are not coincident and are extra-axial. Moreover, the adjustment of the light source to the axis of the lamp condenser can seldom be effected to an unquestionable accuracy. The latter operation, where a filament lamp or other type of radiant of a non-homogeneous or relatively extended form is in use, is not theoretically critical, and in the absence of a lamp diaphragm may be impossible. Where one is fitted the simplest procedure is to close it to a pin-hole and, using a wall or other convenient target some yards away, to adjust the centration until the circle of illumination remains concentric whilst the focus of the lamp is altered. In many lamps adjustment of the focus by moving the condenser is apt to disturb the whole lamp.

The lamp itself centred, it is next necessary to align the lamp and microscope axes. Should the lamp inclination joint be extra-axial, this can be an infuriating process, as both lamp and mirror must be adjusted step by step, and it is presumably to secure alignment with a lamp condenser in use that Oettlé employs the catoptric technique. The writer uses this method in aligning apparatus for microprojection, but has never found it desirable to employ a mirror or filter as the reflecting element. Even should the filter be accurately perpendicular to the microscope axis (a postulate which it would be injudicious to assume or rely on), reflection from a plane surface can only indicate parallelism, and not identity, of the axes.

This can be easily demonstrated by inspecting the lower side of the contracted substage iris, where the image of the lamp filament can be focused in an excentric position although the reflection from the filter is concentric with the lamp iris.

It is true that this is immaterial if the lamp condenser projects a truly parallel beam and the radiant is focused in the object plane by the substage condenser, but as Conrad Beck (1938) showed, a source of finite size cannot initiate such a beam, and in this case the lamp diaphragm ceases to control the size of the field of view.

The writer prefers to use a lamp pivoting about the centre of its diaphragm, the condensing lens lying between this and the bulb, which last is adjusted to focus the beam. In this sytem the contracted iris diaphragm is centred, as described above, by the microtelescope technique, and the lamp inclination then adjusted to centre the radiant, which can be seen if necessary by removing the eyepiece.

The insistence of many recent authors on the accurate axial centration of the mirror has long puzzled the writer. It is clear that the optic axis of the microscope must meet the mirror surface at different points as the latter is progressively inclined, unless the centre of rotation of the mirror lies in its reflecting surface ; this condition is not known to obtain in any existing instrument. If, therefore, the centre of rotation lies on the microscope axis, the latter will cut the mirror centrally only when it is reflecting straight back up the tube.

In any case, eccentricity of the mirror is equivalent only to a slight movement of the surface along the optic axis, and can have no effect on the symmetry of illumination unless the user focuses his lamp on the middle of the mirror and assumes it to be axially centred in consequence.

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12.173 XVI.—THE HEART OUTPUT OF THE CHICK EMBRYO.

By A. F. W. HUGHES (Sir Halley Stewart Research Fellow).

(From Strangeways Research Laboratory, Cambridge.)

TWO TABLES AND FOUR TEXT FIGURES.

IN 1934 Barcroft, Flexner, and McClurkin published their paper on the output of the fœtal heart in the goat, in which, among others, was obtained the important result that the ratio of the blood flow through the heart to the body weight was approximately constant during the latter half of fœtal life.

The method used by these workers was plethysmographic, and was checked against determinations by the Fick method on small adult animals of comparable size. The plethysmographic results were increased by a constant factor of 15 p.c., which represented the difference between them and the results given by the more accurate Fick method.

In this paper are presented some comparable results on the heart output of the chick embryo obtained by a method which might be termed " micrographic plethysmography." The heart output is obtained by the difference between systolic and diastolic ventricular volumes, but these are computed from serial photographs of the beating heart. This method eliminates the inaccuracy due to inertia of the plethysmograph, but, as with the latter, measures only the stroke volume minus the coronary flow and minus the volume entering the auricle during the stroke.

The records are made by taking moving pictures of the exposed heart through a microscope and with an ordinary cinema camera working at the normal speed of 16 frames per second. It is necessary to assume from the subsequent calculations from these pictures that the axis of the heart is in a plane parallel to that of the film, and that the heart is radially symmetrical about this axis.

Technical details.—In stages of development up to and including the third day of incubation the embryo can be photographed by transmitted light. The whole blastoderm can be removed from the egg and transferred to a dish of warm saline ; the heart is sufficiently transparent to trace the outline of the ventricular contents. The ventricle then empties completely at each stroke, and the stroke volume is that of the ventricle itself at full diastole. The microscope was used with a linear magnification of 7·5 times on to the film, the field being 1·4 mm. wide. The illuminant was a 250-watt lamp.

In all other instances the embryo was not freed from the yolk sac, and was illuminated from above. The whole egg contents were transferred to a Petri

dish ; the heart was exposed as quickly as possible and at once photographed, for which a Zeiss telephoto lens of 2·5-cm. focal length was used together with extension tubes between camera and lens. For the 5-day embryo a field of view 4 mm. wide was arranged which, as with the foregoing embryo, was continuously observed during photography through a viewing head containing a half-silvered prism.

For later stages of development a field 14 mm. wide was used, which brought the lens too close to the camera to use the viewing head between them. Accordingly a wire frame of the size of the field was fixed at the plane of focus below the camera and the preparation brought to this position when the exposures were made. The source of illumination for the 4-mm. field was a 100-watt Pointolite lamp focused by an achromatic condenser, and for the wider field a Zeiss low-voltage microscope lamp.

The 5-day embryo needed no further dissection than the pushing aside of the chorio-allantois, the ventral body wall not being closed at this stage. For later embryos it was necessary to incise the sternum along the mid-ventral line, thus considerably increasing the degree of trauma to which the embryo was exposed ; this is reflected in an increasing depression of the heart-rate at later stages.

The photographical equipment used was an " Ensign 16-mm. Kinecam " camera mounted in an apparatus designed for general biological cinematography by the writer and manufactured by Messrs. Unicam Instruments, Ltd., of Cambridge.

When the films had been exposed and processed, portions suitable for computation were selected by preliminary normal speed projection, and then from the single frames either paper enlargements were prepared or projection drawings of the ventricular outline were traced. The final magnification of these enlargements or tracings varied from 49 to 44 diameters.
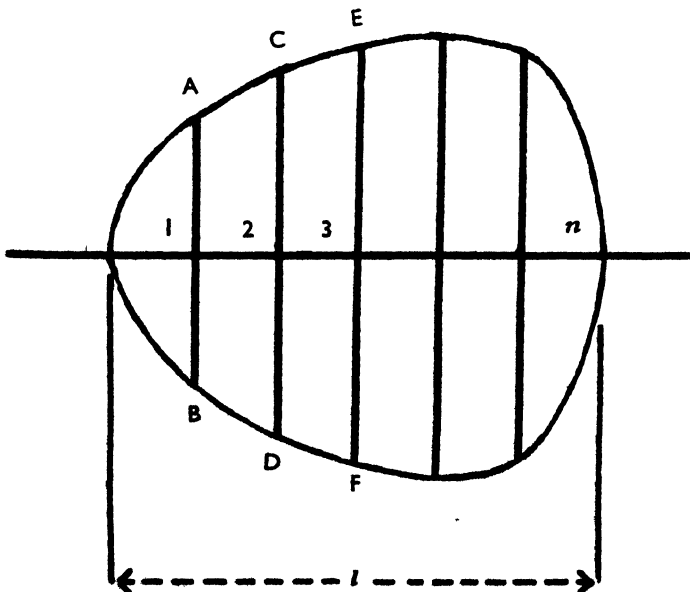
Fig. 1.

CALCULATION (fig. 1).

If *ACE . . . FDB* represents the projection on to a plane of a body radially symmetrical about the long axis, then the volume of the body may be calculated from the projected figure by Simpson's rule.

The length l of the axis of the projected figure is divided into any even number, n, of equal intervals, and on it are erected ordinates *AB*, *CD*, etc., as in fig. 1. If y_1 and y_2, etc., represent the full height of the ordinates, then the required volume is given by the expression :

$$\text{Volume} = \frac{\pi l}{12n} \left[2(y_2{}^2 + y_4{}^2 + y_6{}^2 + \ldots) + 4(y_1{}^2 + y_3{}^2 + y_5{}^2 \ldots)\right]$$

The late Dr. D. E. Lea, of the Strangeways Laboratory, was kind enough to instruct me in the use of this formula. In practice, the figures of ventricular outlines were divided into six intervals along the axis and the necessary measurements made with a scale calibrated in millimetres at the appropriate magnification.
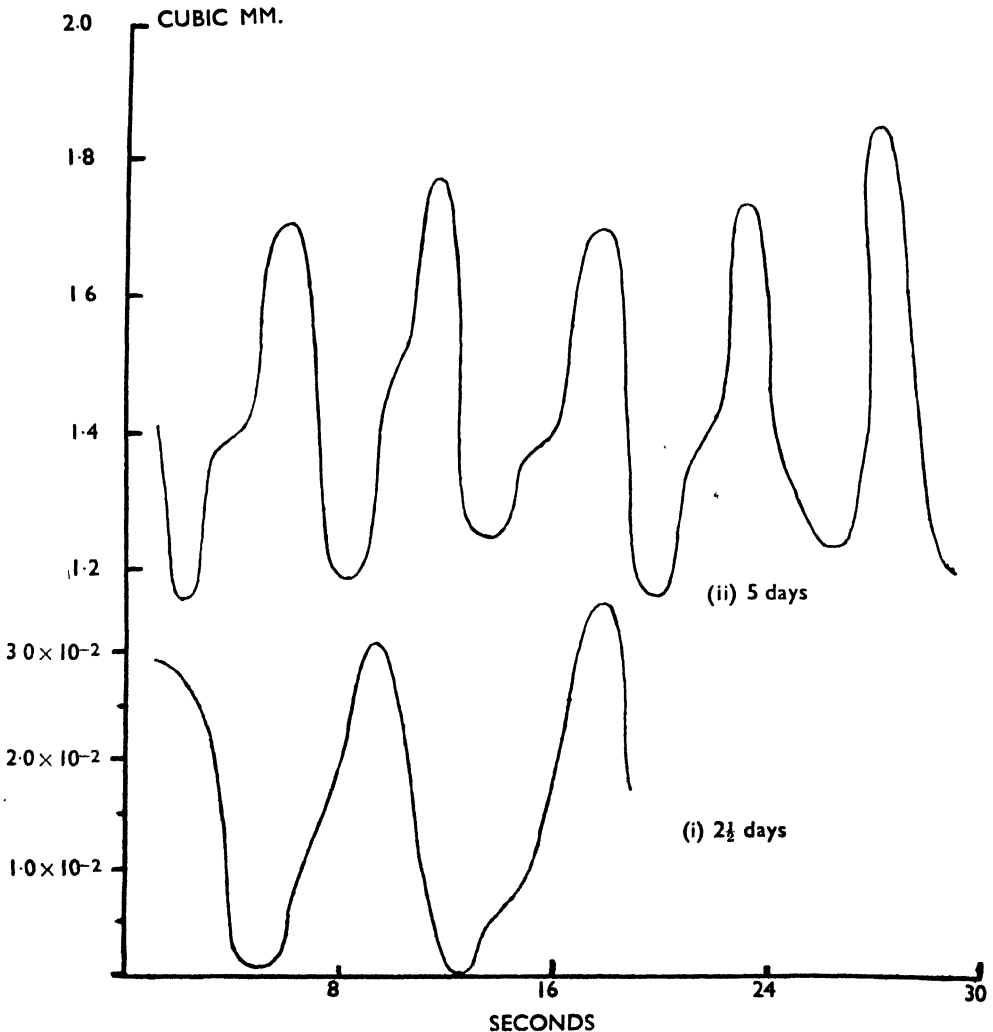
Fig. 2.—Change in ventricular volume with heart beat of early chick embryos. (i) Internal volume of a 2½-day ventricle, (ii) external volume of a 5-day ventricle ; to the separate scales as given.

RESULTS.

The results obtained during the course of this present work are set out in Tables I and II and in text-figs. 2–4. The form of the ventricular volume curves at later stages mostly indicates a rapid contraction at the end of the diastolic period, followed by a slow relaxation. It is uncertain whether the irregularity of the calculated volumes during relaxation is wholly due to scatter resulting from the errors involved in the estimation or whether, in addition, abortive contractions alternate with the effective cycles. Such is suggested by the 17-day curve (fig. 3, iv).
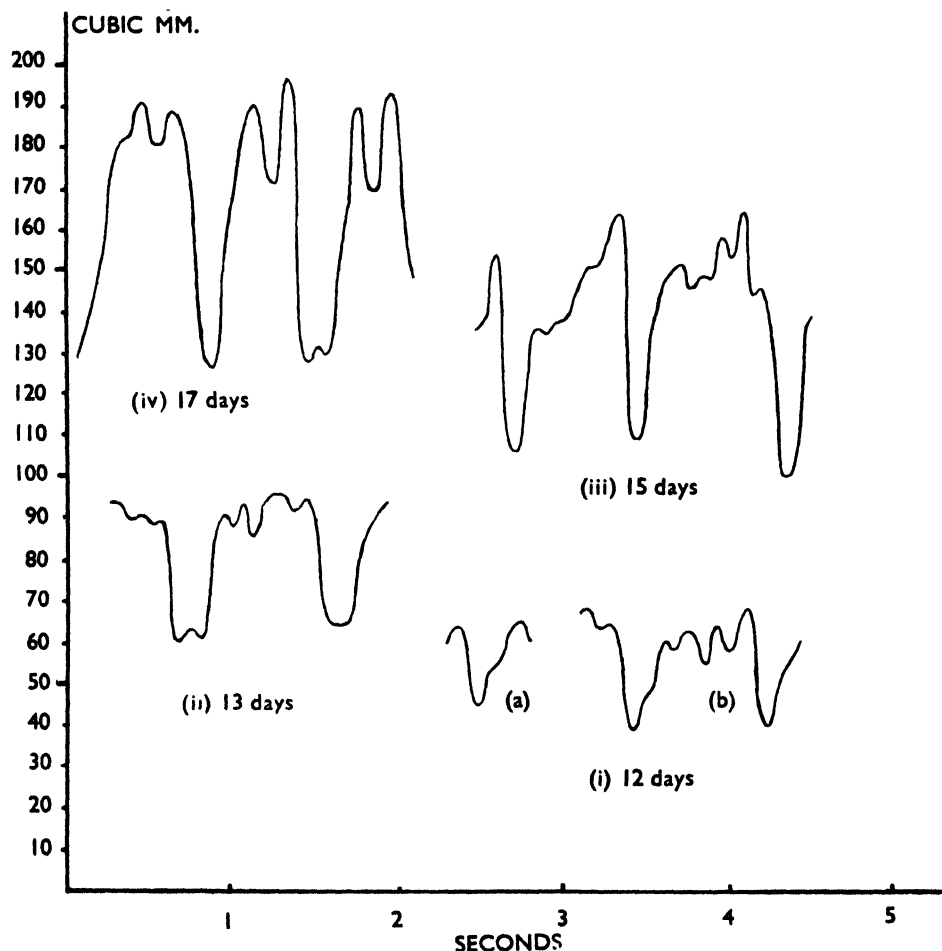
Fig. 3.—Change in external ventricular volumes with heart beat of (i) two 12-day ventricles, (ii) a 13-day, (iii) a 15-day, and (iv) a 17-day ventricle. All to the same scale.

The main objection which can be urged against these results lies not so much in the method itself but in the inherent disadvantage which the chick embryo exhibits for physiological studies of this kind, namely the fact that any manipulation whatsoever considerably depresses the rate of heart beat. Even the act of removing part of the shell of an egg of 3 days' incubation in order to expose the embryo reduces the heart rate by some 25 p.c. The normal range of heart rate for an undisturbed chick embryo is known from the electrocardiographic

studies of Yule Bogue (1932). Yule Bogue's average values have been used as a standard with which to compare those experienced during the present work, and the final estimate of heart output per minute has been obtained by multiplying the measured stroke volume by the average rate of heart beat from Yule Bogue's data. This procedure neglects any possible relationship between rate and volume, but probably only introduces serious errors into the results during the latter half of the incubation period. There is some evidence from the results that the higher proportionate values of stroke volume are associated with the lowest heart rates experienced. For this reason it is considered that the values for the latter half of the incubation period which are here presented probably err on the high side.
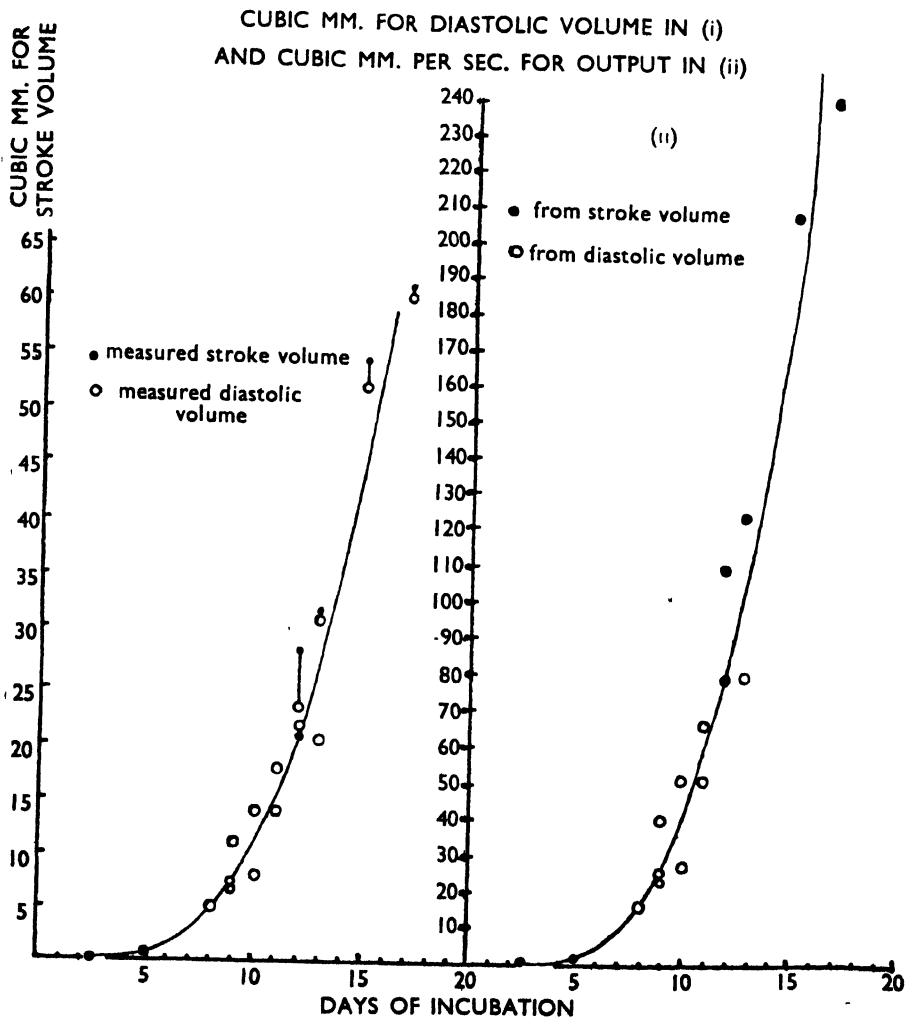
Fig. 4.—(i) Stroke and diastolic volumes of chick embryos during development, to the respectively given scales. Where both volumes were measured from a photographic record, the corresponding points are joined

(ii) Heart output of the chick embryo during development. From the measurements of diastolic volume alone the output has been calculated from the relationship displayed in (i).

From the fifth day onwards the stroke volume bears an approximately constant ratio to the systolic volume, of which it constitutes the high proportion of 30 p.c. In fig. 4 (i) the respective scales for stroke and systolic volumes are based on this proportionality, the closeness of the respective points for these entities for the photographed ventricles shows that values for stroke volume within the general limits of error of the method can be calculated from measurements of diastolic volume alone. This procedure is comparatively easy and rapid, as the slowly beating heart exposed in a late embryo is in diastole for the greater proportion of the cycle (fig. 3). By this means there has been obtained a supplementary series of results for stroke volume and heart output, which are distinguished from the first series in fig. 4 (i and ii).

A corresponding ratio of stroke diastolic volume for the adult mammalian heart can be obtained from the data of Meek and Eyster (1922), who measured the volume changes in dogs' hearts by radiographic means. Their results indicate that the average proportion between stroke and ventricular volume for the adult dog is about 10 p.c.

RELATIONSHIP BETWEEN BLOOD FLOW AND EMBRYONIC SIZE.

In Table I are set out the values for the weight of the embryo and of the embryo and membranes, together with values of the blood flow per minute for unit weight of tissue. The weights of the membranes are taken from Byerly (1930), whose values for the weight of the embryo have been averaged with

TABLE I.

Days of incuba-tion.	Weight of embryo.		Weight of embryo + membranes.		Heart output per min.	Ratio to embryo in ml. per gm.	Ratio to embryo + mem-branes in ml. per gm.
2½	approx.	0·016 mg.	approx.	0·68 mg.	3·6 cu.mm.	225	5·2
5		0·206 gm.		0·706 gm.	0·12 ml.	0·57	0·17
12		5·01 gm.		9·47 gm.	4·8 ml.	0·94	0·506
17		18·34 gm.		24·24 gm.	6·3 ml.	0·34	0·26

those of Murray (1926). The blood flow is given per minute in order to compare these data with those of Barcroft and his collaborators for the goat fœtus.

The data for the 2½-day embryo illustrate the disproportionate size which the circulatory system occupies in the early embryo, and admit of little comparison with the proportionate ratio of later stages, which from 5 to 17 days of incubation are all of the same order of size. In the goat fœtus (Barcroft *et al.*, 1934) for the latter part of fœtal life the quantity of blood flowing per gramme of fœtus is 0·15 ± 0·03 ml. per minute. The values for fœtal goats are therefore rather less than those for the chick, and with less variation between different stages. When the weight of the placenta is also taken into account, the blood flow per unit of embryonic tissue steadily rises. The chick data for embryo plus membranes do not at present point to so clear a result.

The Work done by the Embryonic Heart.

From the values for heart output obtained in the course of the present work and from the values for the arterial pressure of the chick embryo already obtained (Hughes, 1942) estimations of the work done by the embryonic heart have been made.

In computing the work of the goat fœtal heart, Barcroft *et al.* neglected the kinetic energy factor and multiplied blood volume pumped by the heart per minute by the pressure against which it was expelled. This precedent I have here followed.

In dealing with the mammalian embryo, however, it is legitimate to make no distinction between right and left ventricles, as their walls are of equal thickness, and in the rabbit (Hamilton, Woodbury, and Woods, 1937) have been shown to have similar intraventricular pressures. In the chick embryo the thicknesses of right and left ventricular walls are not the same (Hughes, 1943), and therefore a lower pressure in the right ventricle may be inferred. The left ventricle may, of the two, be more responsible for the pressures measured in the chorio-allantoic arteries ; and so on these grounds the values for the work done by the heart obtained by using these pressure values may be too high.

On the other hand, the pressure gradient between heart and chorio-allantoic artery introduces an opposite error into these calculations. An attempt has been made to assess the magnitude of this pressure gradient for a 16-day chick, applying in the Poiseuille formula appropriate values for heart output and aortic diameter. Assuming a blood viscosity of three times that of water, the calculated pressure gradient is about 3 cm. of water per centimetre of dorsal aorta and chorio-allantoic artery, where in the latter the observed pressure was about 35 cm. of water. Thus it is not unreasonable to assume that these two sources of error will appreciably cancel each other, and so to use the chorio-allantoic pressures for the estimation of the energy output of the heart. This has been done for the results in Table II.

Table II.

Days of incubation.	Respiration in cu.mm. per mg. per hour.	Olivo's heart weight in mg.	Corresponding energy in ergs per sec.	Work done by heart in ergs per sec.	Efficiency, p.c.
4	30 (W. & K.)	0·95	$1·6 \times 10^3$	3·5	0·22
6	14·6 (W. & K.)	5·0	$4·1 \times 10^3$	34	0·84
7	15·1 (W. & K.)	8·3	$7·1 \times 10^3$	69	0·97
12	10·0 (? assume)	50·9	$30·0 \times 10^3$	1260	4·2 (?)

The Efficiency of the Embryonic Heart.

In 1927 Warburg and Kubowitz measured the respiratory rate of embryonic chick hearts ; the values they obtained may be used to calculate the energy liberated by glycolysis corresponding to the oxygen absorbed in the usual way. The ratio between this quantity of energy and that appearing as the mechanical work done by the heart is the efficiency.

Warburg and Kubowitz's results refer to chick embryo hearts of 4, 6 and 7 days of incubation; figures for heart output for these stages have been interpolated from the curve in fig. 4 (ii), and the weights of the whole heart have been taken from Olivo's figures (1930). The results are set out in Table II. •

The efficiency of the heart increases with time, partly due to the fact that the work done by the heart increases more rapidly than does its weight; but also due to the decrease in the respiratory rate per unit weight of heart tissue. One general result obtained by the Warburg school on the respiration of embryonic and other tissues is that glycolysis is probably proportional to the rate of growth; the decrease in the respiratory rate of the embryonic heart as development proceeds is therefore mainly due to the decline in growth intensity, and only part of the respiration of the heart bears any relation to its mechanical function. The respiratory rate of adult heart tissue is stated by Lovatt Evans (1939, p. 167) to be 3–4 ml. per gramme weight of tissue per hour, the which is one-tenth the value of that of the 4-day chick heart.

In Table II an estimate of the efficiency of the 12-day chick heart is attempted, a value for the respiratory rate of two-thirds that of the 7-day heart being chosen. On this assumption the progressive rise in efficiency of the heart is maintained, and presumably continues further during later stages of develop-· ment. Rather improbably high values of the efficiency would then result by bringing the respiratory rate of the heart still lower, and towards that of the adult mammal.

SUMMARY.

1. A method is described of estimating the heart output of embryos and small animals by measurements made from cinematographic pictures of the beating heart.

2. This method has been used on the chick embryonic heart from $2\frac{1}{2}$ to 17 days of incubation.

3. The ratio of heart output to embryonic weight is between 0·3 ml. and 1·0 ml. per gramme of embryo between 5 and 17 days of incubation.

4. The work done by and the efficiency of the embryonic heart have been calculated.

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ABSTRACTS

MICROSCOPES AND MICROSCOPY

Photomicrography.—Z. PRICE (" An Auxiliary Microscope Base for use in Photomicrography," *J. Biol. Photog. Assoc.*, **17**, No. 2, 59–63). A microscope base is described which has been designed with the object of enabling a bench microscope to be used for photomicrography in the vertical position. It is massive in order to reduce vibration and contains its own surface-metallized mirror, which eliminates glare due to the multiple images formed in the usual back-silvered mirror. The filter mount arrangements include a water-cell and variable density polaroid filter which is of particular use when photographing in colour. B. O. P.

Phase-contrast Microscopy.—R. MONTARNAL (Contraste de phase en lumière polarisée," *Revue d'Optique*, 1948, **27**, Nos. 8–9, 477–92). A phase-contrast system is described in which a $\pi/2$ phase difference between the direct and diffracted rays is obtained by the use of a phase plate employing quarter-wave mica or cellophane and the object is illuminated by plane polarized light. A development of this system, employing half-wave mica or cellophane in the construction of the phase plate, enables amplitude control to be effected with the aid of a rotatable analyser and positive or negative phase-contrast effects to be obtained. The factors involved in producing maximum contrast in the image are considered both from a theoretical standpoint and in relation to a series of photographs taken at different positions of the analyser. Partially absorbent objects may require a retardation greater or less than $\pi/2$, and for this purpose a Bravais compensator can be used in place of a quarter-wave plate. The object may be illuminated in bichromatic light and the retardation adjusted so that positive phase-contrast effects are produced for the one colour at the same time as negative effects for the other, thus giving enhanced contrast. Other polarized-light phase-contrast systems are reviewed and criticized. B. O. P.

Micromanipulation.—R. BARER and A. E. SAUNDERS-SINGER (" A New Single-Control Micromanipulator," *Quart. J. Micr. Sci.*, Dec. 1948, **89**, Pt. 4, pp. 439–47). Previous types of micromanipulator are reviewed and considered in relation to the features desirable in such an instrument. These include single control, freedom from vibration and backlash, variable sensitivity of control, limitation of movement to the field of view, rapid centration, robustness, and low price. The new instrument, which began as a modification of the Schuster micromanipulator, is described and operating details are given. It has achieved virtually perfect control in two dimensions of a horizontal plane combined with vertical movement controlled by the same handle, and has proved satisfactory with regard to the other features mentioned above. B. O. P.

ELECTRON MICROSCOPY

Aberration Correction.—E. G. RAMBERG ("Aberration Correction with Electron Mirrors," *J. appl. Phys.*, 1949, **20**, 183–6). The possibility of correcting the spherical and chromatic aberration of magnetic electron microscopic objectives by utilizing the opposite sign of the aberration coefficients in "concave" electron mirrors is investigated mathematically. The suggested arrangement is somewhat similar in principle to the metallurgical optical microscope, with a deflecting field in place of the prism in the light case, and instead of the light being reflected by the object, the electrons are reflected by an electron mirror placed behind the specimen. Having passed back again through the objective lens the electrons are further deflected by passing again through the deflecting field into the projector lens. Distortions produced by the deflecting field are corrected by compensating screws in the projector lens. Calculation shows that the required physical dimensions of the "concave" mirror would be of the order of 0·1 mm.; thus the system requires much modification if any practical application of this method of correction is to be made.   C. E. C.

Shadow-casting and Smooth Supporting Films.—R. C. WILLIAMS and R. C. BACKUS ("The Electron-micrographic Structure of Shadow-cast Films and Surfaces," *J. appl. Phys.*, 1949, **20**, 98–106; 3 figs., 13 refs.). The object of the work is to approach more closely the resolution limit of present electron microscopes by improving specimen preparation techniques. The smoothest supporting films are obtained by casting collodion or formvar on glass, and uranium oxide (calculated thickness 6 Å.) is the best direct shadowing material. For pre-shadowing Pd is convenient, but shows slight aggregation. 20 p.c. Pd and 80 p.c. Pt are recommended. Measurement of the shadows cast by suitable Pd-Pt films (calculated thickness about 5 Å.) gives their actual thickness as 75 p.c. of the calculated value. There is a useful appendix on general shadowing techniques.   M. M. B.

Small Spherical Particles of Standard Size.—R. C. BACKUS and R. C. WILLIAMS ("Small Spherical Particles of Exceptionally Uniform Size," *J. appl. Phys.*, 1949, **20**, 224–5; 2 figs.). Small spherical particles with a diameter of 2590 ±25 Å. are found in a polystyrene latex (380G, Lot 3584, supplied by the Dow Chemical Company). Their mean diameter is obtained by an indirect comparison with the optical microscope. They can be used for magnification calibrations and for estimating the local slope in a specimen when used in conjunction with shadowing. They are sufficiently accurately spherical to allow them to be used for estimating the thickness of the shadowing film by comparing their diameter in the direction of shadowing with that at right angles to it.   M. M. B.

Positive Replicas.—C. M. SCHWARTZ, A. E. AUSTIN, and P. M. WEBER ("A Positive Replica Technique for Electron Microscopy," *J. appl. Phys.*, 1949, **20**, 202–5; 6 figs., 5 refs.). A positive replica technique is evolved to permit direct visual interpretation of replicas from metal surfaces. The negative replica consists of a 15 p.c. aqueous latex of polyvinyl alcohol (PVA), the positive of 1 p.c. formvar in chloroform. The PVA can then be dissolved in warm water, and the positive replica is shadowed in the usual way. The PVA can also be used for double stripping.   M. M. B.

Bull Sperm.—L. H. BRETSCHNEIDER ("An Electron Microscopical Study of Bull Sperm: III," *Proc. Koninkligske Nederland. Akad. van Wetenschappen*, 1949, **52**, 301). The electron microscope has been further applied to the study of the bull sperm. The

head-cap has been examined, the distribution of head plasma, and also the chromosomes, the latter by staining, using ammonical silver nitrate in place of fuchsin in Feulgen's reaction. The sperm tail has also been examined. Jensen's spiral body has been clearly demonstrated and also Jensen's ring. By treatment with bacterial enzymes and impregnation with OsO_4 the cortical helix becomes visible. The terminal piece has also been inspected. C. E. C.

Resolution Limit.—O. Schertzer (" The Theoretical Resolution Limit of the Electron Microscope," *J. appl. Phys.*, 1949, **20**, 20–29). This is a general wave-mechanical treatment of the problem of image formation in the electron microscope. The resolving power and contrast are calculated for different conditions of focusing, illumination, and aperture, and it is found that variation in these factors can produce a variation of about 3x in the resolution. It is pointed out that for most specimens, where inelastic scattering is negligible, for perfect imaging there would be no contrast at all, and that in fact the contrast produced is a result of the small aperture and spherical aberration acting on the marginal rays in the objective lens. The problem of contrast is tackled from the contrast in the image of an atom using the Thomas–Fermi model and Boersch's approximations for small and large scattering angles, and it is claimed that the contrast is improved by decreasing the spherical aberration. The methods of correcting spherical aberration are discussed, notably the conducting-foil method. Unfortunately the foil not only provides the support for correcting surface charges but contributes scattering due to its own atoms. The author claims that when a suitable corrective for electron lens aberrations is found, atoms will be visible. C. E. C.

Electron Beams.—Nelson Wax (" Some Properties of Tubular Electron Beams," *J. appl. Phys.*, 1949, **20**, 242–7). A mathematical examination of potential distribution, maximum current density, and beam spread of tubular electron beams. C. E. C.

Multiple Images.—W. S. Grube (" The Effect of ' Multiple Grounds ' on Electron Microscope Images," *J. appl. Phys.*, 1949, **20**, 125). Multiple images may occur when local currents circulate through the microscope chassis and column due to multiple earth returns which are at different potentials. J. F. B.

History of Electron Microscopy.—B. von Borries and F. Ruska (" New Contributions to the History of the Development of Electron Microscopy," *Frequenz*, 1948, **2**, 267–78). A historical review dealing mainly with German work up till 1943. M. M. B.

Refractive Index.—W. Ehrenberg and R. E. Siday (" Refractive Index in Electron Optics and the Principles of Dynamics," *Proc. Phys. Soc., B*, 1949, **62**, 8–21). From consideration of the principles of Fermat and Maupertius the refractive index in electron optics is discussed and the uniqueness of the expression obtained is demonstrated. The use of this refractive index in some electron optical problems is studied and the relationship between the refractive index and the magnetic vector potential is established. J. F. B.

Streptomycin and its Action on the Bacterial Cell as shown by the Electron Microscope.—G. H. Werner (" Sur les modifications apportées par la streptomycine à la structure d'une cellule bactérienne. Étude au microscope électronique," *Compt.*

rend. Acad. Sci. Paris, 1949, **228**, 1260–61). The bacilli ' *Bacillus subtilis* ' were shadowed with gold according to the technique of Williams and Wyckoff (*J. appl. Physics*, 1946, **17**, 23). After a very short contact with 0·5 mgm. per ml. of streptomycin the bacillary body no longer has any definite contour: particles escape from its periphery so that the background of the preparation appears covered with an amorphous substance. With doses of streptomycin of 0·1 mgm. per ml. the bacillary surface shows regular cavities and a granular material lies between the bacilli. The volume of the bacilli is reduced. If streptomycin is added to the culture fluid in which the bacilli are grown some organisms swell up, others contract and the cytoplasm passes out of the envelope, which remains intact. G. M. F.

Electron Microscopy of Brain Tumour Tissue.—H. FERNÁNDEZ-MORÁN ("Examination of Brain Tumour Tissue with the Electron Microscope," *Ark. Zoologi*, 1948, **40**, No. 6, 15 pp., 3 plates). Monomolecular films are of such thickness (below 0·1μ) as to enable the full resolving power of the electron microscope to be used. Protoplasm of the nervous tissue and of brain-tumour tissue shows a great tendency to form monolayers. Cancer cells are more feebly attached to each other than normal cells. Smears of cancer cells and gliomas on thin films of aluminium or beryllium (20–50 Å. thick) enable tumour tissue to be examined while fresh in the electron microscope. An improvement was found to be obtained by inserting a needle into the brain-tumour tissue and then allowing the adhering material to spread on a water surface. The thin sheet of protoplasmic material carries distinct cell nuclei and will be found to have many patches below 100 Å. thick which are well suited to electron microscopic examination. The layer of cytoplasm with nuclei is supported on aluminium or beryllium films in the electron microscope. These thin metal films help to protect the cell contents from the heating effects of the electron beam.

Methods used. (1) In the microsmear method a piece of the tissue is smeared directly upon the object-holder. It is found that the best results are obtained on metal films which for thin smears should be placed in alcohol-ether and then cooled in ice. Control smears for optical microscopic examination are best prepared by preparation on a glass surface, covering with zaponlac, shaving off with a sharp razor and staining. Single cells may be stretched by depositing the fresh smears on zaponlac films lying on a rubber sheet, which is stretched out quickly and fixed until the film is dried. This treatment makes the cells very thin and suitable for electron microscopy. (2) In the surface cell-film method thin glass or metal needles coated with paraffin are inserted into the desired spot of the tissue, drawn out quickly and dipped into distilled water, isotonic saline, or fixation fluid. The tissue covering the needle floats off and can be picked up on the electron microscope object-holders. Patches of varying thickness will be found in the film, which enable all the various structures present to be examined. All kinds of tissue can be prepared in this way. The time allowed for spreading of the films controls the thickness. Microchemical and physical effects can be studied on the films. The films can be strengthened by tanning, by treatment with osmic acid and by formaline. (3) By replica methods using metal or zaponlac microincineration, etc.

Results of applying the above methods. (1) The basic networks of the cell structures are shown up very well, the internal structure of the nuclei of the medulloblasts appears to be characteristic, and to differ from the neuroblast and spongioblast nuclei in medulloblastomas. The polymorphic glioblasts in the glioblastoma multiforme, the commonest brain tumour, show a characteristic vacuolated cytoplasm with encrusted granular masses. The pathological glia cell types which are specific for each tumour differ from the normal glia cells and from each other both in the structure

of the basic network and in the appearance of the granular mass and of the inclusion bodies. (2) The surface cell-film method gives pictures which are true equivalents of the submicroscopic cell structures. All types of glia and ganglion cell cytoplasm reveal a regular network of granules and filaments of spacing 50 to 100 Å. (3) Micro-incineration represents the most effective procedure for such impenetrable objects as the cell nucleus and the larger cytoplasmic inclusions. The empty spaces of the cell spodograms of the light microscope are shown to be filled with submicroscopic particles arranged in a definite way and representing the submicroscopic skeleton of the cell. By insulating the copper supporting grid from the object-holder it can be heated by the electron beam, thus giving controlled incineration of the material in the electron microscope; an electron diffraction picture of the resulting ash yields much information.

<div align="right">A. E. J. V. and K. V.</div>

Electron Microscopy of Granulosis Virus.—E. A. STEINHAUS, K. M. HUGHES, and H. B. WASSER (" Demonstration of the Granulosis Virus of the Variegated Cut-worm," *J. Bact.*, 1949, **57**, 219–24; 3 figs, 5 refs.). An electron microscopic study is presented of the granulosis virus of the variegated cutworm, *Peridroma margaritosa* (Haw.), the only published instance of an insect granulosis in the Western Hemisphere. Other examples of this type of disease have been observed in Europe in larvæ of the cabbage butterfly *Pieris brassicæ* Linn., in the cutworm *Euxoa segetum* Schiff., and in the pine-shoot roller *Cacœcia murinana* Hb. The histopathology of these diseases is characterized by the accumulation of minute granules in the cytoplasm of infected cells. The abnormal opaque white areas of the fat are made up of nodules of hyper-trophied fat cells filled with these granules. With the electron microscope the granules are seen to be oval and approximately 250 by 400 mμ. Occasionally large forms are seen, probably aggregations of small granules. Tissues infected with granulosis virus should be triturated and suspended in a solution containing 0·016 M. sodium carbonate with 0·05 M. sodium chloride for 3–4 hours at 25° C. and centrifuged at 13,000 r.p.m. for 1 hour. The granules then release virus particles, leaving rod-shaped per-forations or cavities in the granule substance. The virus particle is a slightly curved rod 40 by 240 mμ. Several rods may join to form a chain. One granule or " capsule " contains one virus particle.

<div align="right">G. M. F.</div>

The Heart and the Electron Microscope.—B. KISCH, C. E. GREY, and J. J. KELSCH (" Electron Microscopy of the Heart: I," *Exp. Med. Surg.*, 1948, **6**, 346–65; 16 figs., 10 refs.). The appearances of the chorda tendinea and the left ventricle are compared with those seen in diaphragmatic muscle.

<div align="right">G. M. F.</div>

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HISTOLOGICAL AND CYTOLOGICAL TECHNIQUE

Fluorescence Staining with Primulin: a Histological Study in Various Mycoses.—J. C. RADICE and P. NEGRONI (" Coloracion fluorescente mediante la primulina. Estudio histologico en diversas micosis," *Revista Ass. med. argent.*, 1948, **62**, 731–3; 14 figs.). The use of primulin is recommended for use with the fluorescent microscope in determining the presence of mycotic infections. Sections after treating with xylol and the alcohols are brought to water and well washed if fixed in 10 p.c. formol. If the tissues were fixed in a picric acid solution or one containing a heavy metal the section must be washed for half an hour. Sections are treated for 15 minutes with a 5 p.c. solution of phosphomolybdic acid and for 2 minutes with 90 p.c. alcohol, then washed in running water and rinsed in a solution of uranium nitrate. They are then immersed in primulin 1 in 10,000 for 24 hours, washed in tap water, dehydrated, and mounted in Canada balsam. When viewed in ultra-violet light the tissues show no fluorescence, but parasitic fungi such as *Trichosporon proteolyticum*, *Coccidioides immitis*, and *Paracoccidioides brasiliensis* fluoresce brightly.

G. M. F.

Staining Blood Films.—R. H. BLACK (" Hæmoglobin Stains for Use with Thin Blood Films," *Ann. trop. Med. Parasit.*, 1948, **42**, 236–7; 4 refs.). *Cyanol.* The reduced colourless form of cyanol takes on its normal colour in the presence of hæmoglobin. Hæmoglobin in red cells stains cobalt green, and either safranin or Leishman may be used as a counterstain. There is a tendency for the cyanol to wash out of the red cells if the reagents are too acid.

Alizarin Red S and phosphomolybdic acid. After preliminary staining with Ehrlich's acid hæmatoxylin for 15 minutes slides are washed with water and then placed in 10 p.c. aqueous phosphomolybdic acid for 1½ minutes. Another wash with water is followed by staining for 18 hours with a mixture of 50 ml. of a saturated aqueous solution of alizarin red S and 10 ml. of 10 p.c. aqueous phosphomolybdic acid. The slide is washed with water and dried. The hæmoglobin of the red cells is stained bright yellow; nuclei are dark purple.

Reduced fuchsin and methyl green. A stock solution of the leuco-fuchsin is prepared by mixing 1·5 gm. of acid fuchsin, 5 gm. of powdered zinc, 2 ml. of glacial acetic acid, and 100 ml. of distilled water. On boiling the mixture loses its colour; when cool a further 2 ml. of acetic acid is added. To prepare the working solution, 10 m. is filtered off and to this 1 ml. of commercial hydrogen peroxide is added. Thin blood films are fixed with methyl alcohol and dried. The working solution is then allowed to act for 3 minutes. The stain is then tipped off and the slide is counterstained with 0·5 p.c. aqueous methyl green for 1 minute, washed with water, and blotted dry. Hæmoglobin is stained with fuchsin; nuclei are green. G. M. F.

Formaldehyde and Silver Staining.—A. M. LASSEK and M. M. POWERS (" Concentrated Formalin versus a 10 p.c. Solution as a Fixative preceding Silver Staining," *Stain Technol.*, 1949, **24**, 33–7; 3 figs., 5 refs.). In a series of experiments on the brain-stem and spinal cord of the rat, rabbit, cat, monkey, and man, concentrated seems to excel 10 p.c. formalin as a fixative for the staining of axons with silver nitrate or protargol compounds. In several respects concentrated formalin is a better fixative: it has a greater speed of penetration without causing additional distortion or artifacts, the staining time can be considerably reduced, and the normal configuration of the axons, particularly the larger ones, is better preserved. Good results were obtained after 5½ hours' fixation in concentrated formalin. Further, concentrated formalin is at least equal to the 10 p.c. solution in the ease with which paraffin and frozen blocks can be sectioned, in the clearness of the background of stained sections, and in the consistency of the results. G. M. F.

Demonstration of Glycogen.—J. P. ARZAC and L. G. FLORES (" The Histochemical Demonstration of Glycogen by Silver Complexes," *Stain Technol.*, 1949, **24**, 24–31; 4 figs., 6 refs.). Three methods for the histochemical demonstration of glycogen by silver complexes have been reported. In all of them two steps are essential: (1) hydrolysis of the polysaccharide; (2) reduction of the silver by the hydrolysed carbohydrate. Except in the technique put forward by Gomori (*Amer. J. clin. Path.*, 1946, **10**, 177) a third step, " development," is necessary. The following procedure gives satisfactory results. Hydrolysis for 20–30 minutes in 10 p.c. chromic acid; immerse in the following: aqueous silver nitrate 10 p.c. solution 4 ml., saturated solution of lithium carbonate to 20 ml.; drop concentrated ammonium hydroxide until the precipitate is almost entirely dissolved; complete to 100 ml. with lithium carbonate saturated solution; stand and filter. The solution can be kept in well-stoppered glass bottles. Slides can either be placed in this complex for 15 minutes at room temperature, being reduced with 2 p.c. neutral formol for about 30 seconds, or they may be incubated for 50–60 minutes in a 10 p.c. solution of the complex at 45° to 50° C. This is followed by a thorough washing in water. Sections are toned with 1 in 500 gold chloride. Collodion coating and fixation in either formol-alcohol or picro-formol-alcohol-acetic acid are essential for direct reactions. G. M. F.

The Plasmal Reaction.—E. R. HAYES (" A Rigorous Redefinition of the Plasmal Reaction," *Stain Technol.*, 1949, **24**, 19–23; 14 refs.). The question has arisen whether the plasmalogens or acetal phospholipids are stained by the plasmal reaction or whether ketosteroids are also visualized. The plasmal reaction has been modified and made more specific so that only acetal lipids are stained. The two essential points in making such specificity possible are (1) limiting the duration of the action of mercuric chloride for from 2 to 10 minutes, thus ensuring that neither acid hydrolysis nor oxidation plays any part in the unmasking; (2) obtaining negative controls upon sections of the same block of tissue treated in an identical manner except that immersion in mercuric chloride is omitted. Lipids positive under these conditions are acetals, but not necessarily acetal phospholipids. In order to satisfy the second criterion above material must be either unfixed or fixed for only 1–6 hours in formol. Longer fixation in formol both destroys the acetals and unmasks some non-acetal lipids. Glass-rod section lifters must be used to transfer frozen sections. G. M. F.

Pollen-tube Culture.—C. J. BISHOP (" Pollen-tube Culture on a Lactose Medium," *Stain Technol.*, **24**, 9–12; 2 figs., 3 refs.). A modified sugar-agar technique is described for growing pollen tubes. A Van Tieghem cell is used as a moist chamber

and 12 p.c. lactose is substituted for the normally used sucrose. The lactose is not required as a nutrient but as a non-toxic substance which regulates the external osmotic pressure so that water may be absorbed at a slow rate and growth may be achieved without the bursting of pollen tubes. The advantages are: (1) direct observation of germination and growth at any stage and for any period of time; (2) easier prevention of bacterial contamination with the long growth periods required to obtain pollen-tube divisions; (3) a simple method for making permanent slides suitable for cytological study. G. M. F.

American as compared with German Stains.—M. A DARROW, F. KNAPP, E. STOTZ, and H. J. CONN (" A Comparison of American Stains with Recent German Products," *Stain Technol.*, **24**, 1–3). Thirty German stains were compared with American stains. Twenty were essentially similar in staining and spectrophotometric tests to their American analogues. Two, azokarmin G and B, were superior to American stains and three other German stains, toluidinblau, indigokarmin, and safranin were reported as " excellent." Rosanilin, fuchsin, phloxin, and pyronin of German origin were unsatisfactory as stains and the last three were abnormal from the spectrophotometric standpoint. In addition, baumwollblau, wasserblau, fuchsin, Congorubin, rose Bengal, Sudan III, and gentian-violett were abnormal in their colour characteristics but satisfactory as stains. G. M. F.

Demonstration of Red Blood Pigments.—J. HIRSCHLER (" The Selective Demonstration of Red Blood Pigments (hemoglobin and erythrocruorin) in Microscopic Balsam Preparations," *Stain Technol.*, **24**, 13–17; 3 figs., 6 refs.). The following technique allows hæmoglobin and erythrocruorin to be stained intracellularly. Smears and very small animals are fixed for 24 hours at 20°–25° C. in 5 p.c. sulphosalicylic acid in water. Whole invertebrate animals such as *Tubifex rivulorum* are placed in two successive 24-hour baths of 100 p.c. alcohol. Smears are washed in 24-hour baths of 100 p.c. and 95 p.c. alcohol. Whole animals are then dehydrated in 2–3-hour baths, smears in 5–10-minute baths of 90, 80, 70, 60, and 50 p.c. alcohol. The last alcohol is washed out with several changes of distilled water. Small animals are stained up to 20 minutes, smears for 1 minute in 0·1 p.c. aqueous acid fuchsin. Smears and small animals are rinsed in distilled water and transferred to 80 and 90 p.c. alcohols for 10 minutes or less. Dehydration is carried out in absolute alcohol, a mixture of equal parts of 100 p.c. alcohol and xylol, and two changes of xylol. Canada balsam is used for mounting. G. M. F.

Fluorescence Microscopy of Trypanosomes.—S. STRUGGER (" Fluorescence Microscope Examination of Trypanosomes in Blood," *Canad. J. Res.*, Sec. E, **26**, 229–31). A drop of freshly taken blood containing trypanosomes is mixed with a small amount of 1 in 10,000 acridine orange (3, 6-tetramethyldiaminoacridine), made with 0·85 p.c. sodium chloride. The mixture is covered with a cover-slip and examined with a blue-light fluorescence microscope. The microscope can be constructed with a carbon-arc lamp with an attached convex lens to produce parallel rays. The light is filtered with a cuvette (2·5 cm. thick) containing a saturated solution of copper oxide ammonia so that only blue light reaches the plane mirror of the microscope. A filter containing an orange glass is inserted over the ocular: blue light is thus absorbed quantitatively, but green, yellow, and red light can pass unchanged. To obtain an accurate focus a slide with pulverized anthracene is covered with liquid paraffin. Trypanosomes and leucocytes fluoresce with a bright green light, but red cells do not fluoresce. The method can also be applied to dried blood smears. Smears are fixed

for 2–3 minutes in methyl alcohol and stained for 4 minutes in auramine (1 in 100 solution in distilled water with 5 p.c. liquid phenol); washed in distilled water; dried in air; and kept in darkness. Red cells shine slightly as dark green circles and trypanosomes have a bright golden fluorescence in a blue light. The contrast is improved by mounting the slide in a drop of liquid paraffin and covering with a slip. G. M. F.

Cleaning Diatoms.—C. C. SWATMAN (" Cleaning Diatoms for Microscopical Use," *Microscope*, 1949, **7**, 132–6). The cleaning of diatoms takes place in three stages: (1) removal by mechanical means of waste matter, mud, debris, weeds, coarse sand; (2) oxidation of organic matter by acids; (3) breaking up and removal of all remaining insoluble matter by alkalis and mechanical processes. For mechanical separation wire gauze sieves are required, varying from 150 to 300 meshes to the linear inch. The 300-mesh sieve will retain diatoms of about 60 to 70μ in diameter. To break down fossil earth it is first broken into small pieces, crystallized sodium acetate is added and water to make up about 5 p.c. of the quantity of sodium acetate. The flask is heated till the crystals are melted and the liquid begins to boil: ordinary photographic hypo may be used in place of sodium acetate. The process may have to be repeated ten or twelve times. Lime salts are removed by small amounts of dilute hydrochloric acid. Directions are given for removal of iron oxide. G. M. F.

Dehydration by Silica Gel.—S. COLLIANDER (" Silica Gel as a Dehydrator in Microtechnique," *Microscope*, 1949, **7**, 138–9). Silica gel with granules of about 3 mm. is useful as a dehydrating agent. The volume of the granules is about 1400 to 1500 ml./1000 gm. and is not changed when loaded with water. By heating to 120° C. for some hours the water is fully driven off and the silica gel is then reactivated and can be used again. The quantity of water that will be adsorbed by silica gel is about 20–25 p.c. of the weight of the adsorbent: to adsorb 2 ml. of water 10 gm. of silica gel is taken. After heating at 120° C. for 2–3 hours, the granules are cooled in a desiccator with an air-tight cover; after cooling they are stored in well-stoppered, wide-necked bottles of 50 to 200 ml. Absolute alcohol can be prepared by taking 250 gm. of silica gel and adding to it 100 ml. of 96 p.c. alcohol. The well-stoppered container is shaken and allowed to stand for 24 hours. The excess of silica gel allows the alcohol to remain at 100 p.c. for several months. G. M. F.

Fish Embryos.—C. VAN DUIJN, Jr. (" Entire Preparations of Fish Embryos," *Microscope*, 1949, **7**, 136–8). Fish eggs and embryos should be fixed in 32 or 40 p.c. by volume formaldehyde solution for 15–30 minutes. After washing the material is placed is Strasburger-Flemming mixture, which consists of equal volumes of 96 p.c. alcohol, pure glycerol, and distilled water. A few drops of 0·5 p.c. aqueous erythrosine or a saturated alcoholic solution of eosin should be added. Material is kept for at least 24 hours in this solution before being transferred to equal parts of glycerol and 96 p.c. alcohol for the same period. The specimen is then put in pure glycerol for 24 hours and transferred first to a mixture of one part of terpineol and three parts of alcohol, one part of terpineol and one of alcohol, and three parts of terpineol and one of alcohol each for 24 hours. Pure terpineol is then used for 12 hours. The specimen is now washed in phenol-xylene, followed by pure xylene, and mounted in Canada balsam. G. M. F.

" Dispersion Staining."—G. C. CROSSMON (" The ' Dispersion Staining ' Method for the Selective Coloration of Tissue," *Stain Technol.*, 1949, **24**, 61–5; 2 refs.). Sections from formalin-fixed material are mounted under a cover-glass in a mixture of

two liquids such as diethylene glycol monobutyl ether with cinnamaldehyde and examined with the dark-field microscope. The refractive index of the liquid used for mounting must be of high dispersion and equal or close to the index of the specimen. The optical combinations which have given good results are as follows:

Objectives.	Condenser.	Stop size.
10 × apochromatic (16 mm.), 0·30 N.A.	Achromatic 1·40 N.A., top element removed to give 0·59 N.A.	13–14 mm.
20 × achromatic (10·25 mm.), 0·40 N.A.	Ditto	16–18 mm.
43 × achromatic (4 mm.), 0·65 N.A., with funnel stop	Ditto	16–18 mm.
10 × achromatic (16 mm.), 0·25 N.A.	Abbe 1·25 N.A., top element removed to give 0·30 N.A.	16–17 mm.
43 × achromatic (4 mm.), 0·65 N.A., with funnel stop	Ditto	22 mm.

Results were not as good when using the 10 × or 43 × achromatic objectives with the Abbe condenser. Tissue elements, dependent on their refractive index, are coloured in shades of blue, red, or yellow according to whether their refractive index is lower than, the same as, or higher than that of the mounting medium.   G. M. F.

Iron-hæmatoxylin for Intestinal Protozoa.—M. GOLDMAN (" A Single Solution Iron-hæmatoxylin Stain for Intestinal Protozoa," *Stain Technol.*, 1949, **24,** 57–60; 8 refs.). Two solutions are prepared. Solution A is a 1 p.c. solution of hæmatoxylin in 95 p.c. alcohol, prepared by diluting a stock solution of 10 p.c. hæmatoxylin in 95 p.c. alcohol. This solution need not be ripened. Solution B consists of iron alum (ferric ammonium sulphate-violet crystals) 4·0 gm., glacial acetic acid 1·0 ml., conc. sulphuric acid 0·12 ml., and distilled water 100 ml. Mix solutions A and B and in a few hours the colour changes from a rich purple to a dark brown with a purplish cast. The solution should then be filtered and is ready for use. If the colour changes to greenish black the solution is unsuitable for use. In open Coplin jars the stain lasts about 1 week. Smears are fixed in Schaudinn's solution with 5 p.c. acetic acid for 5 minutes at 50–60° C. or for 1 hour or more at room temperature. Wash in iodized 70 p.c. alcohol for 5 minutes; pass through two changes of 50 p.c. alcohol for 3 minutes each. Stain in above solution for 5 minutes. Wash in tap water for at least 5 minutes and if permanent preparations are required for 15–30 minutes. Dehydrate, passing through alcohols and xylol. Mount in Clarite or other neutral mounting medium.
          G. M. F.

Brilliant Cresyl Blue for Chromosomes.—W. N. STEWART and A. N. SCHERTIGER (" Brilliant Cresyl Blue as a Stain for Plant Chromosomes," *Stain Technol.*, 1949, **24,** 39–45; 8 figs., 5 refs.). A 2 p.c. solution of brilliant cresyl blue in 45 p.c. aqueous acetic or propionic acid is used with fixed material in making smear preparations. Staining is carried out for 3–5 minutes and the techniques for staining are similar to those used in the aceto-carmine method. For permanent preparations a mounting medium is made by mixing polyvinyl alcohol 56 p.c., diluted with water to the consistency of thick molasses, with 22 p.c. lactic acid and 22 p.c. phenol by volume. Permanent slides are prepared by floating off the cover-slip of the temporary slide in 70 p.c. alcohol, then applying the mounting medium and replacing the cover-slip. The chief advantages claimed are: the stain is rapidly and simply prepared; the

staining procedure is in some instances shorter than when using aceto-carmine; the stain has a high degree of specificity for nuclear structures and gives better results than aceto-carmine with certain plant structures. A very small number of cells is lost in making the slides permanent, since the slide and cover-slip are run through only one solution before mounting. The mounting medium dries rapidly and this shortens the time required before critical examination of permanent mounts can be made.

G. M. F.

Section-cutting for the Electron Microscope.—D. C. PEASE and R. F. BAKER (" Sectioning Techniques for Electron Microscopy using a Conventional Microtome," *Proc. Soc. exp. Biol. N.Y.*, 1948, **67**, 470–74). Using a modified standard Spencer rotary microtome it is possible to cut sections at 0·2μ. Rat liver is fixed by perfusion with 2 p.c. osmic acid and is doubly embedded with " Parlodion " and paraffin. When the sections are to be cut the face of the block is trimmed so that it is about 1 mm. square. The microtome is modified by the addition of a low-angle incline to the feed mechanism so that each advance is only 0·1 of the calibrated value. For full details of technique the original article should be studied by those interested. G. M. F.

A Simple Rapid Stain for Malaria Parasites.—J. W. WOLFF (" Een Eenvoudige Snelle Kleuring voor Bloedonderzoek op Malariaparasieten," *Nederl. Tijdschr. v. Geneesk*, 1948, **92**, 2834–7). Films are fixed in methyl alcohol and stained with Stévenel's blue for 15 seconds. The stain is rinsed with tap water and an aqueous solution of eosin 1 in 1000 is applied for 15 seconds. After rinsing with tap water the staining with Stévenel's blue and the subsequent rinsing are repeated: the films are then dried. G. M. F.

Panchromatic Staining for Hæmatology and Clinical Cytology. A New Combination of the May-Grünwald and Giemsa Components in a Stain of Rapid Application.—G. ROSENFELD (" Corante panchromatico para hematologia e citologia clinica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rapido," *Mem. Inst. Butantan.*, 1947, **20**, 329–34). This stain is said to give preparations which remain permanent for many years. Azur A 0·342 gm., yellow eosin 0·342 gm., methylene blue 0·286 gm., eosinate of methylene blue 0·53 gm., methanol 1 litre. To a blood smear is added 0·5 ml., which fixes the film in 1–2 minutes. Distilled water, boiled for 10 minutes and used within 1 week, is added in twice the amount of the stain and left for 5 minutes or a little longer. After washing in distilled water the smear is dried rapidly between blotting-paper. If the slide is allowed to remain wet it loses colour. G. M. F.

Staining Nucleoli.—W. W. AYRES (" A Method of Staining Nucleoli of Cells in Fresh, Benign, and Malignant Tissues," *Cancer Res.*, 1948, **8**, 352–7; 4 figs., 28 refs.). Cell suspensions in serum are prepared on a cover-slip which is then inverted over a slide containing a fine precipitate of azure C dye. The nucleoli stain clearly and specifically, whereas the nucleus stains only faintly. Slides with a precipitate of azure C are prepared as follows: solutions of azure C in 0·25 p.c. and 0·5 p.c. concentrations in absolute methyl alcohol are prepared. A drop of one of these solutions is placed on a clean slide and quickly covered with another slide; the stain diffuses between the slides; excess stain is removed from the edges with a cloth. The slides are then pulled apart with a rapid sliding motion in the long axis of the slides. As the alcohol dries a fine precipitate of dye is left. Slides with both concentrations of dye are made and can be stored till ready for use. A small drop of fresh serum is placed

on a cover-slip and fresh tissue is scraped with a scalpel and mixed with the serum. The tip of the scalpel is used for stirring. The cover-slip is then inverted on a stained slide and ringed with vaseline. The nucleoli stain within a few minutes. The nucleolus is dark azure with fine strands of nucleolar material between some nucleoli. The nucleus is unstained. The method can be applied to blood. G. M. F.

Periodate in Bacterial Staining.—D. PENNINGTON ("The Use of Periodate in Microbiological Staining," *J. Bact.*, 1949, **57**, 163–7; 2 figs., 4 refs.). Bacteria to be examined are fixed either by heat, in Bouin's solution, or other fixative. They are then immersed in a 1 p.c. aqueous solution of sodium metaperiodate for 5–15 minutes at room temperature. After washing with water preparations are stained for 15 minutes in sulphite-decolourized basic fuchsin solution (L. C. Coleman, 1938, *Stain Technol.*, **13**, 123), washed for 10 minutes in sulphur dioxide water (5 ml. of 10 p.c. $K_2S_2O_5$ and 5 ml. 0·1 N. HCl in 100 ml. water), washed in water, and dried. Polysaccharide structures in many bacteria are stained red. Hotchkiss (*Arch. Biochem.*, 1948, **16**, 131) discusses the specificity of the reaction. G. M. F.

Polychrome Methylene Blue Staining of Fungi.—E. MUSKATBLIT ("Staining of Fungi in Scales and Hairs: Method of Staining with Polychrome Methylene Blue," *Arch. Derm Syph. Chic.*, **59**, 236—42; 6 figs., 8 refs.). Scales and nail scrapings are broken up into tiny pieces and are pasted on a slide with raw egg white. The preparation is dried in air or over a bunsen burner. Carnoy's fluid for 5 minutes fixes the preparation and removes the fat. After pouring off the fixative the slide is dried in air. Staining is carried out with Loeffler's polychrome methylene blue; afterwards the slide is carefully washed for 1 or 2 minutes in tap water. Decolourizing is carried out with a 1 p.c. solution of acetic acid for 1 or 2 minutes. The preparation is washed in water (2 minutes), absolute alcohol (5 minutes), xylol (5 minutes), and mounted in Canada balsam. Fungi and bacteria stain purple or bluish purple. G. M. F.

Capsule and Mucus Formation by Bacteria.—E. KLIENEBERGER-NOBEL ("Über Kapsel-und Schleimbildung bei Bakterien," *Schweiz. Z. Path. Bakt.*, 1948, **11**, 336–45; 14 figs., 6 refs.). To stain capsules impressions are made on cover-slips from blocks of 15–20 p.c. serum agar by cutting out small cubes with growing cultures on their surface. The cover-slips and blocks are placed in open Petri dishes containing Bouin's fluid for 1–2 hours. The agar blocks are then detached with a knife and the cover-slips after washing are stained with 1 in 20 Giemse's solution for 15–30 minutes. After staining the cover-slips are mounted on a slide in water or neutral glycerin-gelatine. Another method is to treat the cover-slips for 30 minutes in 5 p.c. tannic acid, wash thoroughly, and stain in 1 in 10,000 crystal violet for 15–30 minutes. For mucus staining bacteria are spread on a cover-slip, dried in air, and fixed for 20 minutes in Chabaud's solution, washed in water, and placed for 30 minutes in 5 p.c. tannic acid. After washing preparations are stained for 1 hour in 1 in 10,000 crystal violet and mounted as above. These preparations do not reveal any capsular swelling when pneumococci are treated with immune serum. G. M. F.

Ketosteroids in the Adrenal Cortex.—B. CAMBER ("Histochemical Demonstration of Ketosteroids in the Adrenal Cortex," *Nature*, 1949, **163**, 285–6; 1 fig., 9 refs.). Various methods for staining ketosteroids in the cells of the adrenal cortex have depended on the formation of coloured hydrazones of the ketosteroids. Coloured compounds of greater intensity are produced if the aryl-hydrazones of the ketosteroids are coupled with diazonium salts. Formalin-fixed tissue thoroughly washed in running

water, is sectioned on the freezing microtome. Sections cut at 5μ are allowed to remain overnight in a saturated aqueous solution of 2-hydroxy-3-naphthoic acid hydrazide and washed in N/100 hydrochloric acid and then in distilled water to remove excess reagent, immersed in a solution of 0·4 p.c. sodium hydroxide, and transferred to a dilute solution of the diazonium salt. This may be prepared from a stabilized diazonium salt. If the diazonium salt solution is too·strong, staining tends to be patchy. Sections may then be counterstained, cleaned, and mounted in the usual way. The intensity of the reaction is improved if, before coupling, the sections are put into distilled water to which tincture of iodine is added till a faint straw colour is obtained. After 5 minutes, 1 p.c. sodium thiosulphate solution is added, drop by drop, till the solution is cleared. The distribution of the coloured material in otherwise unstained adrenal tissue is shown in a figure.   G. M. F.

Pleuropneumonia-like Organisms in the Uterus.—I. G. SCHAUB and J. A. GUILBEAU (" The Occurrence of Pleuropneumonia-like Organisms in Material from the Postpartum Uterus; Simplified Methods for Isolation and Staining," *Bull. Johns Hopkins Hosp.*, 1949, **84**, No. 1, 1–10; 15 refs.). The presence of pleuropneumonia-like organisms in the normal and pathological vagina and cervix has been recorded by a number of observers. Evidence is now brought forward that these organisms can frequently be found in the postpartum uterus. They were isolated from 17 of 112 patients and in 11 cases they occurred in pure culture. The incidence was considerably higher from patients who had received penicillin than from those who had not. This finding does not support the theory that pleuropneumonia-like organisms represent variant forms of other bacteria produced *in vivo* by the action of penicillin. Material from the uterine cavity was inoculated immediately into fluid thioglycollate medium containing 0·5 p.c. dextrose and 0·0001 p.c. resazurin. To the medium was then added 0·1 ml. of a sterile 4 p.c. solution of clarase (final concentration 0·25 p.c.) to neutralize penicillin and 2 ml. of sterile ascitic fluid. After 24 hours' incubation and daily thereafter, the original thioglycollate cultures were streaked on plates of pancreatic digest agar containing 20 p.c. ascitic fluid. For staining, a block of agar 5 mm. square is cut from the plate and placed colony side up on a slide. A small 1-mm. loop of Wayson's stain is placed in the centre of the agar block (0·2 gm. basic fuchsin and 0·75 gm. methylene blue in 20 ml. absolute alcohol; add the dye to 200 ml. of 5 p.c. solution of phenol in distilled water; filter). The agar block is surrounded by flour balls of plasticine, approximately 2–3 mm. in diameter and placed on the slide so as to form the corners of a 1-cm. square round the agar block. A cover-glass is lowered on to the stained agar block and the plasticine balls, and the preparation is examined with an oil-immersion lens.   G. M. F.

CYTOLOGY

Embryogeny of *Pherosphæra*.—C. G. ELLIOTT (" The Embryogeny of *Pherosphæra hookeriana*," *Proc. Linn. Soc. N. S. Wales*, 1948, **73**, 120–29). This study was conducted on the Tasmanian species of *Pherosphæra*, *P. hookeriana*. Archer, cones being collected on Mount Mawson in the Mt. Field National Park and material being fixed in formalin-acetic-alcohol prior to dissection and sectioning.

Examination of some 62 cones indicated an abnormally low fertility, despite the close proximity of male and female plants : 75 p.c. of the ovules were abortive, and of the remaining 25 p.c., embryos were found in only 62 p.c.

The proembryo consists of a tier of six to ten pro-suspensor cells and a number of

embryo initials (four to six), arranged in two tiers. Generally the embryo units consist of two uninucleate cells, although units consisting of a single binucleate cell are found. A two-celled unit is formed from a binucleate cell as a result of the laying down of a wall on a cleavage plain, although it is noted that possibly binucleate cells do not always develop into two-celled units, as one embryo unit was observed with four nuclei arranged tetrahedrally, with rudiments of a wall between two of them, indicating formation of a four-celled unit direct from the binucleate cell. That this does occur has been demonstrated in *Podocarpus andinus* by Looby and Doyle, who suggested that this phenomenon is as much a Podocarpean characteristic as are the binucleate cells themselves.

Concerning the occurrence of binucleate cells in developing embryos (mentioned above), as distinct from embryo initials, the author believes this to be the first published record, although he points out that they possibly do not occur in embryos older than those he examined.

Each proembryo develops independently, producing embryonal tubes, by the growth of which they are eventually separated. Since there is an embryo in the terminal position which will become the definitive embryo, embryogeny is of the type which Buchholz termed " determinate cleavage polyembryony." Two embryo units sometimes occur in the lower tier, this condition being somewhat indeterminate.

As in most conifers possessing a prosuspensor tier, what is properly termed a " rosette " tier is absent in *Pherosphœra*, but nevertheless " rosette " embryos do occasionally occur above the prosuspensors, and, as previously suggested by Buchholz, may arise by (1) division of a prosuspensor cell at the upper end or (2) from the relict nuclei or from the open-cell tier.

The author has found no definite evidence of apical cell growth in *Pherosphœra*, as was demonstrated in *Dacrydium* by Buchholz, although in young embryos cells are frequently observed in the position of apical cells.

Discussing the Podocarpean affinities of *Pherosphœra*, it is noted that it resembles *Dacrydium* in the arrangement of the embryo units and also in exhibiting determinate cleavage. The supposed development in embryo initials of two-celled units from binucleate cells points strongly to the Podocarpean affinities of *Pherosphœra*. The author sees in the absence of an epimatium in *Pherosphœra* the culmination of an evolutionary sequence towards erectness of ovule and reduction of epimatium illustrated by species of *Dacrydium*, to which the former seems directly related.

The taxonomic implications of this embryogenic study are discussed, and an interesting diagram setting forth the author's conclusions as to the relationship of the *Pherosphœra* with the Podocarpaceæ and Taxodiaceæ is given. In this *Pherosphœra*, while admittedly differing in certain characters from the Podocarps (a distinctive feature of *Pherosphœra* being the usual occurrence of two-celled embryo initials instead of binucleate cells which are general in Podocarps) is shown as being directly related to *Dacrydium*. The great similarity between these two genera lies in the nature of the cone and fertile branch.

A natural scheme of classification is suggested, based on the nature of the primary fertile branch, and the author further proposes a new sub-family of the Podocarpaceæ, namely the Dacrydioideæ, to include *Dacrydium, Pherosphœra, Microcachrys, Acmopyle,* and the *Microcarpus* sections of *Podocarpus,* together with *Podocarpus vitiensis* and *P. minor.* This sub-family would be characterized by the richly branched habit associated with retention of the complete fertile branch system, and by the fertile branches having a well-developed basal vegetative portion in which the leaves are typically keeled and imbricating.

Discussing the significance of the two-celled embryo units and binucleate cells in

Pherosphœra, the author suggests that one cell of the two-celled unit represents the cell which elongates to form the primary suspensor in some conifers (*Chamæcyparis, Biota,* and *Sciadopitys*), and that the absence of a primary suspensor in Podocarpaceæ is correlated with the occurrence of binucleate cells dividing into four-celled units, the two courses being mutually exclusive. E. D. H.

DIATOMS AND ALGÆ

A Bed of Diatomaceous Earth in Anatolia.—E. ALTINLI, and L. R. IRMAK (" Geyikçeli (Kayseri) Diatomiti. A Note on Diatomaceous Earth from Geyikçeli (Kayseri Vilâyeti, Turkey)," *Rev. Fac. Sci. Univ. Istanbul,* 1946, **11**, B, 131–4). The occurrence of a bed of diatomaceous earth among Neogene volcanic and lacustrine strata in Anatolia is reported. The deposit, which consists almost entirely of *Melosira* sp., with a few sponge spicules, appears suitable for commercial exploitation. It is of considerable extent, the total amount available being estimated at 8,500,000 cubic metres. R. R.

New Fossil Diatoms.—J. W. BARKER, and S. H. MEAKIN (" New and Rare Diatoms," *J. Quekett Micr. Club,* 1945, Ser. 4, **2**, 18–22, pls. 3–4). Ten new species of fossil diatoms from the deposits of Moreno in California, Singiliewsky in Russia, Barbados, Oamaru in New Zealand, and Java are described and illustrated by photo-micrographs. In connection with one of these the authors discuss the correct applica-tion of the generic name *Eupodiscus* Ehrenberg, which they consider should be confined to those diatoms possessing projecting processes close to the margin but no distinct furrows or rays. In addition, two species of *Entogonia* originally described by Greville are figured, one, *E. Davyana* Grev., showing the curious tubular " canals " characteristic of the genus. These are very rarely found attached to the valve owing to their great fragility. R. R.

New Fossil Diatoms.—J. W. BARKER and S. H. MEAKIN (" New and Rare Diatoms," *J. Quekett Micr. Club,* 1946, Ser. 4, **2**, 76–9, pl. 15–16). A further ten new species of fossil diatoms are described and figured in this paper. They come from the Moreno Shale in California, from Singiliewsky and Carlovo in Russia, from Barbados, and from Oamaru in New Zealand. A complete frustule of *Pyxilla Johnsoniana* Greville is also figured, and the two valves shown to be very dissimilar, the lower one being that described as *Pyxilla ? reticulata* Grove & Sturt. *Rattrayella Churchi* Hanna is renamed *Eupodiscus vallatus* with no justification for the dropping of the original epithet, and a description and figure of it are given. R. R.

Freshwater Algæ from the Azores.—P. BOURELLY, and E. MANGUIN ("Contribu-tion à la flore algale d'eau douce des Açores," *Mém. Soc. Biogeogr. Paris,* 1947, **8,** 447–500, pls. 1–8). This list of 434 freshwater algæ from the Azores is preceded by a discussion of the origin and age of the algal flora of the islands. The suggested means by which freshwater algæ might have arrived there are migratory birds, man's activities, by the wind, and by ocean currents. Of these, birds are probably the most important and account for the presence of a number of arctic species and some tropical forms. The diatom flora contains a rather high proportion of endemics—almost 19 p.c.— but most of these are classed as forms, not even varieties, of well-known cosmopolitan species. In other groups there are only about 4 p.c. of endemics. This suggests that the colonization of the islands by freshwater algæ occurred comparatively recently,

geologically speaking, as was to be expected from the recent volcanic activity. The algæ listed include 36 *Cyanophyceæ*, 89 *Conjugales* (most of them desmids), 47 *Chlorophyceæ*, and 244 diatoms. Of these 1 form of *Cyanophyceæ*, 1 species of *Euglenophyta*, 1 species, 2 varieties and 2 forms of *Chlorophyta*, and 1 variety of *Conjugales* are described as new.   R. R.

Bacteria-free Cultures of Marine Diatoms.—S. P. CHU (" Note on the Technique of making Bacteria-free Cultures of Marine Diatoms," *J. Mar. Biol. Assn. U.K.*, 1946, **26**, 296–301). The author gives details of the methods which he has found useful in making bacteria-free cultures of marine diatoms. It is advisable first to obtain a flourishing crude uni-algal culture, which is sub-cultured several times in artificial sea-water at low temperatures and in bright light. This reduces bacterial growth greatly. Selected single cells are then washed, using micro-pipettes, through a series of drops of artificial or Berkefeld-filtered natural sea-water. These are then inoculated into each of the media—artificial sea-water, nutrient sea-water broth, sea-water agar, nutrient sea-water agar. The last of these proved a very suitable medium for keeping bacteria-free cultures, since bacterial growth is good and hence any contamination rapidly becomes visible. Diatom growth is also good. A medium consisting of baked sea-mud powder covered with a layer of natural sea-water is recommended as a good medium for starting crude uni-algal cultures of marine diatoms.
                                                                                R. R.

Australian Diatomites.—I. CRESPIN (" A Study of Australian Diatomites with special reference to their Possible Value as Filter Media," *Bur. Min. Resources, Geol., Geophys. Australia, Bull.*, 1947, No. 7, 1–40). All known deposits of diatomite in Australia, including Tasmania, are listed. They number 76, all freshwater, of which 46 have been examined in detail. Their extent and diatom content are described. Ten different assemblages of diatoms are recognized, six from tertiary deposits and four from recent or sub-recent ones. The tertiary assemblages are dominated by *Melosira* or *Synedra*, whilst the recent ones consist mostly of neritic forms. The paper concludes with a discussion of the possibility of using some of the diatomites for filtration purposes. A small number, including examples from Victoria, Tasmania, South Australia, and West Australia seem suitable.   R. R.

Freshwater Diatoms from Devon.—J. FALKNER (" Freshwater Diatoms, Devon, Supplementary List to that of Bessell (1909 and 11)," *Trans. Torquay Nat. Hist. Soc.*, 1946, **9**, 191–7). The first part of this paper consists of a list of 124 species and varieties of freshwater diatoms not previously recorded in Devon. This is followed by a list of 56 species and varieties found in roadside ditches around Torquay in habitats which are dry for part of the year. The paper concludes with a note on an abnormal form of *Eunotia* and a similar abnormality in *Meridion circulare* Ag., found in moorland pools.   R. R.

Diatoms from Streams in Funen.—N. FOGED (" Diatoms in Water-courses in Funen. I. Stavis Aa (Stavis Brook)," *Dansk Bot. Arkiv*, 1947, **12**, 5, 1–40). N. FOGED (" Diatoms in Water-courses in Funen. II. Lindved Aa (Lindved Brook), III. Odense Aa (Odense Brook)," *Dansk Bot. Arkiv*, 1947, **12**, 6, 1–71). The diatom flora of five streams in the Danish island of Funen has been investigated and the two papers above record the results for three of them. The topography of the area in which the streams run is described in some detail. The greater part of their courses

runs through comparatively flat agricultural land, but their upper reaches are in more hilly and boggy ground. At a number of stations, varying from eight to fifteen on the different streams, collections of diatoms were made on from one to nine occasions, and at the same time the air and water temperature, the pH, oxygen content, bicarbonate content, and chloride content were recorded. The diatom material was acid-cleaned and one strewn slide from each gathering was made. These were traversed systematically until about 1000 valves had been identified and counted. The species were classified according to their position in the halobion spectrum, Boye Petersen's allocation to the various categories being followed for the most part. In the Lindved Aa and the Odense Aa the chloride content varies between 20 mg. and 60 mg. per litre, rising towards the mouth, and the same is true of the upper reaches of the Stavis Aa. About half-way along its course, however, this stream receives the flow from a salt well whose water contains almost 6000 mg. of chloride per litre. Immediately below this influx the chloride content of the water in the stream is 130 mg. per litre. It soon falls to 80 mg. per litre and remains at that figure for most of its course. The majority of the diatoms in all three streams belong to the oligohalobous-indifferent group, their percentage rarely falling below 70 p.c. and being usually over 80 p.c. Oligohalobous-halophobous, oligohalobous-halophilous, and mesohalobous species are all found, but usually in small quantity. The occurrence of each of these is considered in some detail and a revision of the ecological status suggested in some cases. In general the halophilous and mesohalobous forms are found in greater numbers in the lower reaches of the streams. The majority of the diatoms in the salt well were mesohalobous. R. R.

A Rare Diatom from Uruguay.—J. FRENGUELLI (" *Nitzschia* (*Nitzschiella*) *ventricosa* Palmer neritica en el litoral del Atlantico del Uruguay," *Not. Mus. La Plata*, 1945, **10**, 139–42, pls. I–II). This rare diatom was discovered in a gathering made from a mat of coralline algæ occasionally exposed by unusually low tides at La Paloma on the Atlantic coast of Uruguay. The previous literature relating to the species is reviewed. It has been found in coastal gatherings from various places in the tropics. A detailed description of the species, illustrated by photomicrographs, is given.
R. R.

Diatoms from South America.—J. FRENGUELLI (" *Melosira* (*Aulacosira*) *patagonica* (O. Muell.) Freng. a proposito de una critica de la Doctora A. Cleve-Euler," *Not. Mus. La Plata*, 1946, **11**, 91–9). The author reviews the history of this species, first recorded from Tierra del Fuego by P. T. Cleve as *Melosira* sp. in 1900 and next by O. Müller from Patagonia as *Melosira lineolata* Grun. var. *patagonica* O. Muell. In his previous publications Frenguelli had recorded it from a number of recent and fossil gatherings from the southern tip of South America, had pointed out that Cleve's and Müller's records referred to the same species, and has discussed its systematic position in some detail, pointing out that it was to be united neither with *Melosira lineolata* Grun. nor with *Melosira Roeseana* Rabenh., as Cleve had suggested, but must be considered as a separate species. It was also found in South Chile by Krasske, who said that it was probably only a variety of *Melosira Roeseana* Rabenh. Frenguelli had contended against this view in a recent publication. A. Cleve-Euler had also found the species recently in Lake Frey in Patagonia and said of it, " I do not think this species so closely related to *M. Roeseana* as does Mr. Frenguelli." This misrepresented Frenguelli's views entirely, as he regards the species as completely separate, and a very interesting example of a diatom with a very limited distribution.
R. R.

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Diatoms from Jugo-Slavia.—F. HUSTEDT (" Diatomeen aus Seen und Quellge-
bieten der Balkan-Halbinsel," *Arch. f. Hydrobiol.*, 1945, **40**, 867–973). Collections of
diatoms were made in various localities in Jugo-Slavia in 1934. On examination of
the material 441 forms belonging to 346 different species were found: 49 species,
4 varieties, and 2 forms were new and are described in German and figured. The
majority of the species are members of the Central European flora; 10 were forms of
alpine distribution; 1 had previously been found only in the Arctic; and 4 have a
predominantly tropical distribution; 59 are not known living from localities outside
the Balkans. The diatom flora of Asia Minor is practically unknown, however, and the
possibility that some of these species are to be found there also is pointed out. A
larger number of the endemic species were found in Lake Ochrid and its neighbourhood
than anywhere else, and on the basis of the occurrence in that lake of a few species
previously known only from a pre-Pleistocene deposit from the Rumanian Carpathians,
the author suggests that it contains a relict flora. A striking feature of the diatom
flora of the Balkans as seen in these gatherings is the high proportion of *Navicula* spp.
and the small numbers of *Eunotiæ* and *Pinnulariæ*. This is to be explained on ecological
grounds, the waters of the area being rich in carbonate owing to the prevalence of
limestone in the underlying rocks.   R. R.

Structure of Diatoms.—F. HUSTEDT (" Die Struktur der Diatomeen und die
Bedeutung des Elektronenmikroskops für ihre Analyse," *Arch. f. Hydrobiol.*, 1945,
41, 315–32). It has been established by investigations with the optical microscope
that the valves of many species of diatoms consist of two layers, one with fine pores or
poroids, the other with much larger openings. These are separated by a mesh-work
of vertical walls so that the valve consists of a series of chambers with a large, usually
single, opening on one side and a sieve-plate on the other. On the basis of electron
microscope studies a very different interpretation of the structure for *Pleurosigma
angulatum* (Quek.) W. Smith has been put forward by Müller and Pasewaldt (*Die
Naturwiss*, **30**, 1942). They consider that this diatom has two layers separated by
pillars only, not walls dividing the intervening space into chambers. One membrane
has a series of elliptical sieve-plates in it and the other a series of slit-like openings
corresponding in number and position. The apparent slits are considered by Hustedt
to be interference phenomena, this view being based on their extreme regularity, on
observation of the parallax effects they show in stereo pictures, and by their alteration
in direction with alteration in the angle of cut of the lines of markings. The conclusion
is that the large elliptical openings and the sieve-plates are at two levels, not one, and
that the membranes in which they lie are separated by walls, not pillars. The structure
is thus similar to that of coarser forms.   R. R.

Diatoms in N. German Lakes.—F. HUSTEDT ("Die Diatomeenflora norddeutscher
Seen mit besonderer Berücksichtigung des holsteinischen Seengebiets. I–IV. Seen in
Posen, der Neumark, Pommern, und der Mark Brandenburg," *Arch. f. Hydrobiol.*,
1945, **41**, 392–414). A short account of the characteristics of each of sixteen N.
German lakes and a list of the diatoms found in each is given. Most of the gatherings
consist either of plankton or of mud from comparatively deep water, and the littoral
flora is accordingly inadequately represented. *Stephanodiscus astræa* Ehrenb. is the
most widespread and abundant species. *Fragilaria crotonensis* Kitt. is almost as
common in Pomerania, but less frequent elsewhere. *Melosira granulata* (Ehrenb.)
Ralfs was only occasionally found in quantity, but *Melosira islandica*, subsp. *helvetica*
O. Müll., occurs in a number of the lakes. It flourishes in oligotrophic conditions
whilst the ecological requirements of *Melosira granulata* (Ehrenb.) Ralfs are more
eutrophic waters.   R. R.

British Soil Diatoms.—J. W. G. LUND (" Observations on Soil Algæ. I. The Ecology, Size, and Taxonomy of British Soil Diatoms," *New Phytol.*, 1945, **44**, 196–219; 1946, **45**, 56–110). The diatom floras of 66 soils from a variety of habitats, gardens, and allotments predominating, were examined. The top 2 cm. of soil was removed in the field. In the laboratory samples were removed by scraping portions of the soil surface with the sharpened edge of a triangular needle. Seven mounts were made from each soil, as much as possible being mixed with sterile water without hindering observation, and five longitudinal transects were made over each preparation, using a $\frac{1}{16}$-in. objective and a ×10 eyepiece. The various species present and their abundance were noted. After this examination the material from two slides was washed on to nutrient agar plates, one was placed in a moist chamber and examined at intervals during the following week, and the material of the other four was acid-cleaned and made into permanent preparations. A further sample of soil was placed in a petri dish with 0·05 p.c. Benecke solution. The cultures were examined at intervals over about a year. In addition the pH, base deficiency, organic matter, carbonate, phosphate, nitrate, and available potassium contents of the soil were determined.

The base-deficient soils, which were all acid and generally poor in nutrients, had low productivities and few species. The remaining soils, which possessed better supplies of nutrients, had a richer and more diverse flora. The presence or absence of certain species was controlled by the base status of the soil, some only occurring on the more calcareous soils, a few only on acid soils, and others mainly on more or less neutral soils. The diatoms were most frequent in the upper layers of the soils, the uppermost 1 mm. being usually richer than any other layer. Seasonal variation was studied in one garden soil which was kept free from weeds. Spring and autumn maxima were distinguished, but they were not very marked. It is suggested that in natural conditions the changes in vegetative cover throughout the year would result in much greater variation in diatom productivity.

The prevalence of small diatoms is pointed out. Most belong to species which are small when growing in aquatic habitats, but soil specimens are often smaller than those from water. With small size go changes in form which may be summarized as follows:

(i) The apical axis decreases in length relatively more than the transapical and pervalvar axes;

(ii) the outline of smaller forms is less complex and more evenly rounded;

(iii) the density of valve markings increases, although not in proportion to the reduction in size;

(iv) in forms whose central and terminal striæ are differently oriented, those on the smaller valves are more nearly parallel.

The bearing of these observations on taxonomy is discussed. The advantages of small size in a soil habitat are thought to be the greater freedom of movement and ability to appose one side to the water film on soil particles, which may assist in the uptake of water and salts. It will also favour dispersal in soil dust. On the other hand, the higher surface-volume ratio means more rapid water loss when the soil dries. The changes in shape with decrease in size, however, reduce considerably the increase of this ratio.

In the taxonomic part of the paper 52 species are described, of which 7 are new, as are also 7 varieties. The descriptions are all in English. Most of the forms mentioned are figured and measurements are given in every case, together with notes on occurrence. Often also there are critical discussions on their taxonomy. This part of the paper, and particularly the figures, will be of great value to future workers on soil and aerial diatoms. R. R.

The Cilia of Diatoms.—A. A. C. E. MERLIN ("Cilia the True Cause of Diatom Movement," *J. Quekett Micr. Club*, 1945, Ser. 4, **2**, 26–8, pl. 5). This note is illustrated by some photographs which purport to show cilia surrounding diatoms stained with silver and with fuchsin. The cilia are described as close-packed and forming a halo around the specimens. The preparations were made by A. C. Coles and an outline of the methods which he is understood to have used is given. These are the normal bacteriological versions of the techniques employed. The argument is advanced that, since these methods do not show false flagella in bacterial preparations, the cilia demonstrated must be real.   R. R.

Diatoms and Desmids from the Bernese Oberland.—E. MESSIKOMMER ("Algen aus dem westlichen Berner Oberland (Quellgebiet der Simme), *Mitt. Naturf. Ges. Bern*, 1945, n.f., **2**, 75–106). In a series of gatherings from lakes and streams between 1050 m. and 2500 m. in altitude in the western Bernese Oberland, 350 algæ, including 174 diatoms and 94 desmids, were found. One species and two varieties of diatoms and one variety and one form of desmid are described as new. Compared with their proportions in the neighbouring low-lying regions, the diatoms are over-represented in the flora, and this is attributed to the height and to the low humus content of the waters investigated. *Cyanophyceæ* are also well represented with 29 species. The absence of soft water in the region, together with the poverty in humus, limits the desmid flora and accounts for the preponderance within it of species of the genus *Cosmarium*. In the highest lake from which samples were taken, the Upper Wildhornsee at 2497 m., the only species of algæ found were the two diatoms *Hantzschia amphioxys* (Ehrenb.) Grun. and *Surirella ovata* Kütz. This lake lies adjacent to a glacier and the author concludes that diatoms are the first algal settlers in alpine localities.   R. R.

Diatoms and Desmids from the St. Galler Oberland.—E. MESSIKOMMER ("Beitrag zur Kenntnis der Algenflora der Gewässer im Gebiete der Grauen Hörner (St. Galler Oberland)," *Vierteljahrsschr. Naturf. Ges. Zürich*, 1946, **91**, 237–53). In twenty-four gatherings from lakes and streams in this part of Switzerland, 406 algæ, including 246 diatoms and 104 desmids, were found. The very high proportion of diatoms is explained by the fact that they are suited to alpine conditions and humus-poor waters, and also by some of the samples coming from running water. The genera *Diatoma, Neidium, Pinnularia, Cymbella, Gomphonema*, and *Nitzschia* are particularly well represented among the diatoms, as is *Cosmarium* among the desmids. There is no falling off in the number of species found in individual localities below 2300 m., for, although many species do not occur above 2000 m., others which reach their maximum in sub-alpine or alpine conditions compensate for this. In the rigorous conditions at high altitudes in waters ice-bound for 10–11 months of the year and with low water temperatures always, only small forms are to be found. At high altitudes the composition of the algal flora is less influenced by chemical factors than it is at lower levels, and physical factors are more important.   R. R.

Diatoms from Uruguayan Peat.—F. C. MUELLER-MELCHERS ("Diatomeas procedentes de algunas muestras de turba del Uruguay," *Com. Bot. Mus: Hist. Nat. Montevideo*, 1945, **1**, 17, 1–21, pls. I–II). The diatom content of five samples of peat from various localities in Uruguay have been examined: 74 species were found, and 17 of these, 18 varieties, and 5 forms are new records for Uruguay. The dimensions and density of striation of the specimens of each species are recorded. One sample was dominated by *Diploneis subovalis* var. *argentina* Freng. and another by *Epithemia*

Zebra (Ehrenb.) Kütz. The others had no dominant species. A striking feature of these peats was the frequency of the *Chrysostomataceæ* accompanying the diatoms.

R. R.

Diatoms from Lakes and Streams in Pennsylvania.—R. PATRICK ("A Taxonomic and Ecological Study of Some Diatoms from the Pocono Plateau and Some Adjacent Regions," *Farlowia*, 1945, **2**, 143–221, pls. 1–3). This paper is based on the study of some sixty gatherings from lakes and streams in a region in north-east Pennsylvania consisting mainly of late Devonian and early Carboniferous sandstone rocks with a few local limestone outcrops. Most of the gatherings were squeezings from submerged vegetation, either flowering plants or mosses, but there were also a number of scrapings from rocks, etc. The greater part of the paper is devoted to a taxonomic account, with dichotomous keys, of the 248 species, varieties, and forms found. These include six new species and five new varieties. There are critical discussions of the identity of many of the species mentioned and of the previous literature referring to them. Latin diagnoses are provided for the new species and varieties. In the ecological account the pH, bicarbonate, and calcium content of a number of the lakes from which gatherings were made are given and the most common species in each mentioned. For the most part the mineral content of the waters was low and their pH below 7. Species of *Eunotia* were the most prominent constituent of the vegetation. The association of certain species with particular physical habitats, i.e. dripping rocks, partially submerged moss and fast-running water is noted. Attention is also drawn to the fact that the few species of *Nitzschia* found all occurred in gatherings from alkaline waters associated with limestone outcrops. Unfortunately determinations of the chloride content of the waters were not available and hence the occurrence of the species could not be correlated with this factor and comparisons made with the findings in European waters of Kolbe, Boye Petersen and Hustedt. R. R.

Diatoms from a Texas Bog.—R. PATRICK ("Diatoms from Patschke Bog, Texas," *Notulæ Naturæ*, 1946, **170**, 1–7). Samples of peat taken at 1-ft. intervals from the surface to a depth of 22 ft. in this bog were investigated. An equal weight was taken from each level and acid-cleaned, the residue made up to 10 c.c., thoroughly agitated, and 0·2 c.c. deposited on a cover-glass. Quantitative comparisons were thus made possible. Seventeen species and varieties, including two new species of *Pinnularia*, were found, all at depths between 2 ft. and 5 ft. The 4-ft. and 5-ft. levels contained many *Pinnulariæ* including some suggesting a comparatively cold climate. In the 3-ft. level *Desmogonium guianense* Ehrenb., which occurred sparingly at 4 ft., was common. This is a tropical and sub-tropical species and its presence suggests a warmer climate at the higher level, a conclusion which is in agreement with the results of pollen analysis. R. R.

Algæ from the Kamtchatka Hot Springs.—J. BOYE PETERSEN ("Algæ collected by Eric Hulten on the Swedish Kamtchatka Expedition, 1920–22, especially from Hot Springs," *Kgl. Danske Vid. Selsk., Biol. Medd.*, 1946, **20**, 1, 1–122). The greater part of the material examined in this investigation came from hot springs, and a general consideration of the algal and particularly the diatom flora of such springs is presented. Contrary to observations in America and Europe, the hot springs of Kamtchatka were found to have a flora whose halobion spectrum showed that it did not resemble those of brackish water. It is suggested that the previous observations were made on thermal waters which had a high chloride content, whereas the Kamtchatka hot springs are no richer in chlorides than normal fresh waters. The literature relating to the

temperatures which algæ can withstand is then reviewed and compared with the observations made in Kamtchatka, which are shown to be consistent with those reported previously. The composition of the diatom flora of the Kamtchatka hot springs is compared with two lists of diatoms from Icelandic hot springs and one from a hot spring in the East Indies. It is found that none of these lists contain species not found in cold waters in the same region and that there are only two species which exhibit high constancy and high frequency in the thermal waters of the three regions. The conclusion is drawn that there are no special thermal diatoms and the diatom flora of hot springs is composed of eurythermal species from the cold-water flora of the surrounding region. In the systematic part of the paper 38 species of *Cyanophyceæ*, 195 species of *Diatomaceæ*, including 6 new, together with 2 new varieties and 2 new forms, and 22 species of *Chlorophyceæ* are listed, particulars of their occurrence in the samples examined given, and, for diatoms, their place in the halobion system stated. R. R.

Diatoms in the Paleocene and Lower Eocene.—TH. REINHOLD (" Het Voorkomen van Diatomeën houdend Palaeoceen-Eoceen," *Verh. Geol.-Mijn. Gen. Nederl. Kolon. Geol. Ser.*, 1945, **14**, 391–401). This is a brief review, well documented, of the recorded occurrences of diatoms in the Paleocene and Lower Eocene. Beds containing well-preserved diatom frustules in quantity are always associated with volcanic activity and are usually found interspersed with ash layers. Examples of these from the period under discussion are found in Denmark, the Volga basin in Russia, north-west Germany, and Franz-Josef Land. Poorly preserved diatoms in lesser abundance from the same period have been found in the London Clay in England, the Ypresian of Belgium and northern France, from the same formation in borings in Holland, and from clay dredged in the North Sea. North Africa and Argentina have also yielded fossil diatoms from this period. R. R.

Diatoms from Portuguese Freshwater Deposits.—A. A. DA SILVA (" Diatomáceas fósseis de Portugal, Jazigos de Rio Maior, Óbidos e Alpiarça," *Bol. Soc. Geol. Portugal*, 1946, **6**, 1–166). After a few pages devoted to generalities about diatoms and diatomite and a useful review of the previously published work on Portuguese diatoms, a systematic account of all the species found in the freshwater fossil deposits listed in the title is given. In all, 139 species and 18 varieties were found. Each of these is described in Latin and figured and a comparatively full synonymy is given. De Toni's antiquated systematic arrangement is used, but Hustedt's views on the delimitation of genera and species are followed. Many of the species are new to Portugal and many others have not previously been reported there or elsewhere as fossils. All the deposits are freshwater, but those of Rio Maior show evidence of having been laid down close to estuarine waters which at times invaded the area. There are in the deposit a number of species characteristic of saline waters, such as *Navicula Johnsonii* (W. Smith) O'Meara and *Pleurosigma angulatum* (Quek.) W. Smith. Many of the valves in the Rio Maior deposits have been fractured, presumably as a result of tectonic influences. These deposits are very uniform in composition. The Óbidos deposits are much more varied in composition and their diatoms are less fractured. It would seem that they were laid down in different conditions and at different dates. Some are formed exclusively of *Cyclotella* spp., all planktonts, whilst others consist chiefly of littoral forms such as *Cymbella* spp. and *Eunotia* spp. The Alpiarça deposits contain much more detritus than those from the other localities and a somewhat different flora. R. R.

Auxospores in *Bacteriastrum*.—R. SUBRAHMANYAN ("On the Formation of Auxospores in *Bacteriastrum*," *Curr. Sci.*, 1945, **14**, 154–5). This short account of the formation of auxospores in *Bacteriastrum varians* Lauder gives no cytological details. The valves of a single cell move apart and the protoplast, covered by a delicate membrane, the perizonium, emerges. It grows considerably, then contracts from the perizonium on one side and secretes a valve, repeating the process for the opposite valve. The characteristic setæ are developed at the same time as the valve. The perizonium is ruptured and vegetative divisions, leading to the formation of a new chain or cells, follow. The diameter of these cells is about $2\frac{1}{2}$ times that of the original filament. R. R.

Cytological Life-history of *Navicula halophila*—R. SUBRAHMANYAN ("On Somatic Division, Reduction Division, Auxospore-Formation and Sex Differentiation in *Navicula halophila* (Grunow) Cleve," *Curr. Sci.*, 1945, **14**, 75–7). This short note summarizes the cytological life-history of *Navicula halophila* (Grun.) Cleve. In somatic division mitosis is normal and the chromosome number is 48–52 ($2n$). At the beginning of auxospore formation two cells secrete a common mucilaginous envelope around themselves. The nucleus of each cell undergoes a normal meiosis and when it is completed the protoplast divides. Each of the four haploid nuclei then divides mitotically, one daughter nucleus in each protoplast degenerating. Each of the conjugating frustules now contains two gametes. Both those from one cell escape from the valves and fuse with the two gametes of the other cell, which remain passive within the parent frustule. Thus both gametes of one cell are physiologically male and both those of the other physiologically female and the species may therefore be considered as diœcious. This condition has not previously been observed in diatoms. R. R.

Mitosis and Cytokinesis in S. Indian Diatoms.—R. SUBRAHMANYAN ("On the Cell-division and Mitosis in Some South Indian Diatoms," *Proc. Indian Acad. Sci.*, 1945, **22**, 331–54, pl. 29). A detailed account of mitosis and cytokinesis in five species of diatoms is given. The species investigated were *Terpsinœ musica* Ehrenb., *Triceratium dubium* Brightw., *Biddulphia mobiliensis* Bail., *Achnanthes inflata* (Kütz.) Grun. and *Pleurosigma angulatum* (Quek.) W. Smith. Various fixatives and stains were tried, but PFA_3 proved to be the best fixative and iron alum hæmatoxylin the most satisfactory stain. Permanent preparations were made with these using the protozoological smear technique. Aceto-carmine preparations were also made and rendered permanent by the method given by Lee. Material of *Terpsinœ musica* Ehrenb. was also embedded and cut in sections of 20μ thickness. Feulgen's reaction was used both on the resting nucleus and in the early stages of mitosis. This showed that the nucleolus did not contain chromatin, but positive reactions were given by the reticulum and the chromosomes. The process of mitosis was similar in all the species examined. No centrosomes could be detected and the spindle was barrel-shaped and blunt-ended. It appeared in early metaphase and its origin could not be detected. The nucleolus disappeared at the end of prophase, but the nuclear membrane frequently persisted into metaphase in *Terpsinœ musica* Ehrenb. and *Triceratium dubium* Brightw. No early anaphase figures were seen in the fixed material and mitosis was watched in living material in *Terpsinœ musica* Ehrenb. and the separation of the chromosomes was seen to take place with great rapidity. Cytokinesis was also watched in living material of *Terpsinœ musica* Ehrenb. and *Triceratium dubium* Brightw. It begins at late anaphase and is very rapid, taking about $3\frac{1}{2}$ minutes in *Terpsinœ musica* Ehrenb. and only about 30 seconds in *Triceratium dubium* Brightw. In the former it proceeded

with greater rapidity as far as the spindle, which seems to offer considerable resistance. In *Triceratium dubium* Brightw. and *Biddulphia mobiliensis* Bail. the daughter protoplasts separate considerably as soon as cytokinesis is complete. Secretion of the silicious valve by each of the daughter protoplasts follows almost at once. In *Terpsinœ musica* Ehrenb. it takes from 3 to 4 hours to complete. R. R.

Microspores in Centric Diatoms.—R. SUBRAHMANYAN (" On the Occurrence of Microspores in Some Centric Diatoms of the Madras Coast," *J. Indian Bot. Soc.*, 1946, **25**, 61–6). A number of cases of microspore formation observed in the course of studies of the plankton of the south-west part of the Bay of Bengal are recorded. The forms in which this phenomenon was observed were *Coscinodiscus* sp., *Biddulphia mobiliensis* Bail., *Chætoceros Lorenzianus* Grun., *Actinocyclus Ehrenbergii* Ralfs, *Bellerochea malleus* (Brightw.) Van Huerck, and *Cerataulina Bergonii* Per. There is no previous account of its occurrence in the last three genera. The spores were in all cases rounded and contained a few chromatophores. No cilia were seen. In number they varied from 8 to 64 in each cell. The process of division from the four-celled stage was observed in hanging-drop cultures in two of the species. Although kept under observation, the spores were not seen to be liberated, but after a time degenerated and died. The observations contribute nothing to the solution of the conflict of opinion on the nature of diatom microspores—whether they are part of the life cycle or are a parasite.
R. R.

Plankton Diatoms from the Madras Coast.—R. SUBRAHMANYAN (" A Systematic Account of the Marine Plankton Diatoms of the Madras Coast," *Proc. Indian Acad. Sci.*, 1946, **24**, 85–197, pl. II). Each of the species found by the author in plankton gatherings made along the Madras coast of India is described and figured. Short synonymies and a list of references to previous figures are also given. 143 species were found, of which 9 are described (in English) as new, as are 4 new varieties. The flora shows considerable resemblance to that of the Java Sea, about 50 p.c. of the Madras species having been found there. On the other hand very few of the forms recorded by Karsten from the " Valdivia " collections from the deep-water areas of the Indian Ocean were found. Many of the species had not previously been recorded from Indian waters. R. R.

The Size of Diatoms.—R. S. WIMPENNY (" The Size of Diatoms. II. Further Observations on *Rhizosolenia styliformis* (Brightwell))." *J. Marine Biol. Assn.*, 1946, **26**, 271–84). The author records the results of diameter measurements of individuals of *Rhizosolenia styliformis* Brightw. in many gatherings, mostly from the North Sea, but including some from other waters around Britain, from the Antarctic, and from other seas. The taxonomy of the species is considered and it is divided into a type form, apparently confined to the eastern N. Atlantic and particularly common in the North Sea, a var. *semispina* Karst. of wide distribution in coastal and continental shelf waters, and a var. *oceanica* Wimpenny widely distributed in the deeper oceans. It is thought that the var. *semispina* Karst. may grade into *Rhizosolenia hebetata* Bail., but more material is required before the point can be decided. The type variety formed auxospores in the south-west Dogger swirl area in 1932–3 and again in 1936–7. The generation giving rise to auxospores was about 50 microns in mean diameter and the auxospores had a mean diameter of about 100 microns. There were revivals of the numbers of the 1932–3 generation in the winter of 1936–7 and again in June 1937. These were associated with periods of low temperature and high salinity, whilst the new wide generation arose in the spring and summer of 1936 in a period of low salinity.

The indication that high salinities favour smaller forms is found in the fact that the diameters of individuals from the Faeroe–Shetland area are less than those recorded in the Dogger swirl area, and those from stations south-west of the British Isles are smaller still. In the Faeroe–Shetland area auxospore formation is an annual event.

R. R.

Various Forms of a Diatom in Culture.—D. P. WILSON (" The Triradiate and Other Forms of *Nitzschia Closterium* (Ehrenberg) Wm. Smith, forma *minutissima* of Allen and Nelson," *J. Marine Biol. Assn.*, 1946, **26**, 235–70). The behaviour of this diatom in culture, in which it has been kept at Plymouth and elsewhere since 1910, is described. It is a very feebly silicified species and very plastic in form. Four types of cells occur in the cultures, normal ones with two arms, triradiate with three, cruciform with four, and oval with none. The cruciform type was very rarely found. All types were found to arise from one another and to be able to revert to any other. No canal raphe could be seen with any certainty, this being attributed to the delicacy of the frustule, but there were indications that in the triradiate form it lay along two of the arms. The cell contents include one, two, or occasionally, in triradiate forms, three chromatophores, and also some globules of a fatty nature. The oval type of cell is formed from the armed types by the loss of the arms during division, the new valves having none. They can give rise again, often after many generations, to normal forms by the gradual growth of the arms. Triradiate types arise from ovals which become triangular, but this happens rarely. At times, however, the triradiates so produced are more vigorous than normals and come, by their more rapid division, to dominate the cultures. Whilst normals can apparently only give rise to triradiates by way of ovals, triradiates can, by the gradual shortening and eventual disappearance of one arm, give rise to normals direct. The conditions favouring the production of the various shapes were not determined, but it seems that the production of forms with more than two arms is favoured by good cultural conditions. Reversion of triradiates to normals by the shortening of one arm occurs most frequently in the autumn.

The maximum length of normal cells in culture shows gradual variation over long periods. It is clear that in this diatom the normal cycle of gradual diminution in size followed by auxospore formation and sudden increase does not occur. No trace of auxospore formation has been seen. The silicification is so feeble that the individuals are apparently capable of a small amount of growth, and it is this property which permits the plasticity of the species.

The identity of the form under discussion is considered and it is suggested that it may be specifically separable from *Nitzschia Closterium* (Ehrenb.) W. Smith. It is pointed out that the triradiate form, which has been found in nature on a number of occasions, has been described as *Phæodactylum tricornutum* Bohlin. R. R.

Diatoms from a Pleistocene Deposit near Rome.—V. ZANON (" Giacimento di farina fossile nella bassa valle dell'Aniene ' Sedia del Diavolo ' (Roma)," *Comm. Pont. Acad. Sci.*, 1944, **8**, 425–42). A list is given of 122 species and varieties of diatoms found in a fossil deposit near Rome, which apparently dates from the middle Pleistocene and was laid down in a small lake. There is 2 m. depth of pure diatomite composed almost entirely of *Fragilaria brevistriata* Grun. overlaid by 3 m. of more calcareous deposits with a considerable and varied diatom content, but also containing much clay and sand. In this layer some species characteristic of marine and brackish waters are found, and it is suggested that during a dry period the water in the lake acquired a high concentration of salts, particularly those of magnesium, by evaporation. This produced the conditions in which the diatoms of saline waters were able to colonize the lake. R. R.

Diatoms in a Calcareous Tripoli near Rome.—V. ZANON (" Elementi d'acqua dolce e marina in un calcare farinoso nella zona della ' Sedia del Diavolo ' in Roma," *Acta Pont. Acad. Sci.*, 1947, **11**, 133–48). This note lists 207 diatoms found in a calcareous tripoli occurring in the near neighbourhood of that discussed in the paper above, but lying at a higher level. It contains a few definitely marine species of diatoms and also some marine silico-flagellates and sponge spicules. These are considered to be redistributed from earlier deposits of marine origin eroded by the river Aniene. One new species and one new variety were found in the deposit. Both of them belong to the genus *Caloneis*. R. R.

PROCEEDINGS OF THE SOCIETY

THE ANNUAL GENERAL MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, JANUARY 21ST, 1948, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the President.

New Fellow.—The following Candidate was balloted for and duly elected Ordinary Fellow of the Society:

   L. R. Sadler.   Gidea Park.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

D. H. Browning.	Sittingbourne.
J. Bunyan.	London.
T. Keeling.	Wigston Fields.
G. W. Midgelow.	Manchester.
P. D. F. Murray.	London.
R. R. E. Perkins.	East Grinstead.
H. Polkinhorne.	London.
K. Powell.	London.
Joan H. Tabor.	North Harrow.

ONE HUNDRED AND EIGHTH ANNUAL REPORT.
REPORT OF THE COUNCIL FOR THE YEAR 1947.

The Report of the Council for the year 1947 was read by the Secretary:—

This latter year has been notable for a considerable increase in the activity of the Society and fresh departures reported hereunder. The relaxation of travel facilities greatly helped towards this end and resulted in record attendances at several meetings. Close co-operation with other scientific societies shows signs of bearing fruit.

LOYAL ADDRESS.

On the occasion of the marriage of Her Royal Highness the Princess Elizabeth to His Royal Highness the Duke of Edinburgh, a loyal address was sent to His Majesty the King, which was graciously acknowledged on behalf of His Majesty.

FELLOWS.

During the year 1947 the Council reported with profound regret the deaths of the following Fellows:—

J. B. Capel.	Elected 1913.
H. Price.	„ 1933.
Chetwynd Palmer.	„ 1937.
F. C. Lowe.	„ 1922.
C. J. Pound.	„ 1892.
R. S. W. Sears.	„ 1917.

Ten Fellows have resigned and twenty-nine have been removed from the Roll of Fellowship under By-law 31. Two reinstatements have taken place and fifty new Ordinary Fellows and one Honorary Fellow have been elected. These figures show a gain in Fellowship of seven.

MEETINGS.

Eight ordinary meetings of the Council have been held and eight ordinary meetings of the Fellows. Attendance has been high in all cases.

Following the meeting of March 19th, 1947, the Society held a two-day Conference with the Electron Microscope Group of the Institute of Physics. This proved to be a notable success.

OFFICES OF THE SOCIETY.

In December, by an amicable arrangement with the British Medical Association, initiated by the Hon. Secretary, Mr. F. C. Grigg, the Society relinquished the use of the two offices on the first floor of B.M.A. House in exchange for the exclusive use of the Pillar Room on the ground floor. This move resulted in a much more convenient arrangement of the furniture and equipment, and the general business of the Society has benefited. Our thanks are due to the staff of the B.M.A. in assisting with the transfer of the furniture and fragile effects, none of which sustained any damage.

JOURNAL.

Two numbers of the Journal, Vol. LXIV (1944), parts 3 and 4, and Vol. LXV (1945), appeared during 1947.

Owing to the fact that during the war years the Journal did not appear and to preserve continuity of publication, it has been necessary to describe the last of these numbers as a volume in itself.

During the coming year it is hoped to publish numbers for 1946 and 1947 and to resume the normal quarterly publication for the current year.

The Journals have contained a number of papers dealing with recent developments in microscopy and allied subjects.

Thanks are due to Dr. G. M. Findlay, *C.B.E.*, for his continued labour in office as the Editor of the Society's Journal.

LIBRARY.

During the year 1947 considerable progress has been made with cleaning the books, checking the books on the shelves and dealing with the war-time accumulation of journals. A considerable number that were in need of repair have been attended to and this work will continue in the present year. A part-time assistant librarian has been employed for this work. This work of checking and overhauling the library is a prelude to reorganizing it on a more rational system.

INSTRUMENTS AND APPARATUS.

The following instruments and apparatus have been added to the Society's Historical Collections and the thanks of the Fellows have been duly conveyed to the donors of these valued accessions:—

Exors. of the late Mr. J. Rheinberg—
>Rheinberg's original differential colour screen substage devices.
>A 14,000 lines-per-inch grating replica with a ridged surface.

Mr. W. Williamson, F.R.S.E., F.R.M.S.—
>A small 6-volt micro-slide projector.
>A collection of plain and coloured lantern slides of Mites.

Brigadier H. G. Smith, *C.B.*, *O.B.E.*, *M.C.*, F.R.M.S.—
>An old stage prism illuminator.
>A substage cylindrical lens with stop.
>An old rack-focusing body-tube (Culpeper type) with four objectives.

Mr. F. C. Grigg, F.L.S., F.R.M.S.—
>Smith and Beck Microscope with accessories in mahogany case.

Mrs. L. E. Bowell—
>Large Ross binocular stand with accessories in mahogany case.

Mr. E. B. T. Ogilvy—
>H. Powell type microscope with accessories in mahogany case.

Brigadier H. G. Smith, *C.B.*, *O.B.E.*, *M.C.*, F.R.M.S.—
>Cuff type microscope by Dolland complete with accessories, including Solar Microscope and full instructional diagram and illustrations (*c.* 1780).

One microscope was purchased for use at Sectional Meetings.

SLIDE CABINETS.

The following additions have been reported:—

Mr. D. J. Scourfield, I.S.O., F.L.S., F.Z.S., F.R.M.S.—
>Four slides of *Daphnia Lumholtzi* Sars., originally mounted by C. R. Rousslet; being in a partially dried condition, these were re-mounted into seven slides by the Hon. Sec.

Dr. C. Tierney, F.R.M.S.—
>200 mineralogical slides from the coal measures.

Mr. N. Ingram Hendy, F.L.S., F.R.M.S.—
>40 mineralogical slides.

The thanks of the Society were accorded to the donors.

One hundred and twenty-seven micro-slides and 48 lantern slides have been borrowed from the Society's collections and duly returned.

Out of the original collection by Rousslet of 200 Rotifer slides only 78 were found to be of any use, 56 of these have been re-mounted by the Hon. Secretary; some were divided into more than one.

One hundred and twenty-two slides from the various sections have been repaired and re-ringed and 48 other slides of various subjects have been re-mounted by the Hon. Secretary.

A drawer catalogue with sectional lists is being compiled for the purpose of producing a comprehensive catalogue suitable for publication.

VISITS TO OTHER INSTITUTIONS.

The Hon. Secretary reports that he has visited sixteen other institutions, hospitals, etc., on official business of the Society during the past year; twenty-seven separate visits were made in all.

APPENDIX.

BIOLOGICAL SECTION.

The Biological Section held its usual seven meetings in the Hastings Hall during the year. The attendance ranged from eighteen to fifty-eight, with an average of thirty-one, which indicates that the unfortunate effects of the war years are wearing off, and that interest in the application of the microscope to biological problems is once again gaining ground. The communications maintained a high level without loss of the general informality which characterizes the Section's meetings, and much healthy discussion was stimulated thereby.

Owing to the present catering difficulties it was found impossible to arrange visits to the laboratories of other Societies and Institutions during the year.

INDUSTRIAL SECTION.

The 1947 Session has seen the re-opening of the Industrial Applications Section of the Society. This has been renamed the Section of Industrial Microscopy and it is hoped that it will provide a useful outlet for the inclusion of papers of particular interest to microscopists engaged in industrial laboratories and trade research associations.

The first meeting of the Section was held on October 15th, 1947, and took the form of an Exhibition of Industrial Microscopy. The Council were most grateful to the trade research associations and manufacturing concerns who co-operated to make this show really successful. A large number of visitors saw the Exhibition and from subsequent remarks by Fellows it is felt that this show should become an annual event.

Meetings of the Section are held on the last Wednesday of each month and we have already had papers from Fellows on topics which have been well received. In November Mr. Wredden read a paper on the Microscopy of Enamelled Wire and for the December Meeting the Secretary of the Section gave a general talk on the Microscopy of Paper.

It is hoped that as many Fellows as possible will attend these meetings, which have a general as well as a specialized field. The Secretary hopes that many of the papers which are read at the meetings will be published in the Journal. Arrangements are being made to provide abstracts in the Journal which will be gathered from the trade and scientific press.

On the motion of Mr. C. C. Swatman, seconded by Mr. H. Gunnery, the following resolution was carried unanimously:—

" That the Annual Report be received and adopted."

Vote of thanks to retiring Council.—A hearty vote of thanks was tendered to the retiring officers and members of Council for their continued services during 1947, at the proposal of Mr. W. H. Mannering, seconded by Mr. F. V. Welch.

New Council.—The President appointed Mr. H. Weeks and Mr. B. Verdcourt to act as Scrutineers of the Ballot for the election of Officers and Members of Council for

1948; subsequently, upon receipt of the Scrutineers' report, the result of the ballot was declared from the Chair as follows:—

President.—R. J. Ludford, Ph.D., D.Sc.

Vice-Presidents.—F. C. Grigg, F.L.S., H. Moore, D.Sc., A.R.C.S., J. A. Murray, M.D., F.R.S., E. Wilfred Taylor, *C.B.E.*, F.Inst.P.

Hon. Treasurer.—S. R. Wycherley.

Hon. Secretaries.—F. J. Aumonier, M.Sc., M.R.C.S., D.A., J. Smiles, A.R.C.S.

Ordinary Members of Council.—F. D. Armitage, F.R.P.S., F.L.S., R. J. Bracey, F.Inst.P., L. P. Clarke, *T.D.*, M.R.C.S., D.P.H., G. M. Findlay, *C.B.E.*, M.D., D.Sc., F. Greenshields, B.Sc., Ph.D., H. Gunnery, A. F. W. Hughes, M.A., Ph.D., Maxwell Knight, *O.B.E.*, F.R.E.S., F.Z.S., F.R.G.S., R. Ross, M.A., H. G. Smith, *C.B.*, *O.B.E.*, *M.C.*, *T.D.*, T. E. Wallis, D.Sc., F.I.C., Ph.C., J. M. Watson, A.R.C.S., D.Sc.

Hon. Editor.—G. M. Findlay, *C.B.E.*, M.D., D.Sc.

Hon. Curator of Scientific Collections.—F. C. Grigg, F.L.S.

Hon. Keeper of Instruments.—H. G. Smith, *C.B.*, *O.B.E.*, *M.C.*, *T.D.*

Hon. Keeper of Slides.—Lord Charnwood.

Hon. Librarian.—R. Ross, M.A.

Curator Emeritus of Records.—C. Tierney, D.Sc.

On the motion of the President a hearty vote of thanks was accorded to the Scrutineers for their services.

The President then delivered his Presidential Address entitled " Relation between Cellular Structure and Functional Activity."

The Secretary moved a vote of thanks, which was carried unanimously.

Announcement.—The President made the following announcement:—

The Biological Section will meet in the Hastings Hall on Wednesday, February 4th, 1948, at 5.30 for 6.00 p.m., when the following paper will be read:—

Mr. W. B. Lawrie—
   " Foundry Dust."

Mr. E. D. Hollowday, F.R.M.S.—
   " The Plankton Rotifera of the Tring Lakes."

The Proceedings then terminated.

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XVII.—THE DORSAL ROOT GANGLION CELL OF THE KITTEN WITH SUDAN DYES AND THE ZERNICKE MICROSCOPE.   535.826.7: 576.311.34

By J. Brontë Gatenby and Tohamy A. A. Moussa.

(From the Department of Zoology, Trinity College, Dublin.)

Six Plates and Four Text-Figures.

Introduction.

Ciaccio (1910) described vesicular lipid bodies ("spheroid complexes" of Baker * and Thomas). We believe that these bodies, and the mitochondria, exist separately from the relatively large Golgi apparatus. In our opinion the validity of the silver and osmic picture of the neurone can be upheld. In this present short contribution we intend to describe the cells of the dorsal root ganglion.

Our study of the vertebrate neurones has convinced us that the descriptive cytology of these important cells is not by any means plain sailing. No one could recognize this more clearly than we do, and the present paper sets forth some of these difficulties.

Previous Work.

The majority of histologists have accepted the conception of the neurone set forth in text-books of histology and cytology. There always has been an idea that the Golgi apparatus is a canalicular system. The Parat hypothesis

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* Baker (1944) quotes Ciaccio and considers it sufficient to mention Ciaccio's demonstration of the sudanophility of the Golgi apparatus of mammalian spermatids. It is curious that Baker and Thomas (1948) have not noted that their "spheroid complexes" were demonstrated by Ciaccio in 1910. We trust that this paper and one forthcoming in *La Cellule* will have given Ciaccio proper recognition.

13   185

began in 1924 and flourished for about ten years.   Many studies on neutral red granules in neurones and other cells were published, and in 1929 the present senior writer tried to reconcile the " vacuome " hypothesis and the conventional appearance of the Golgi net by assuming that the argentophile loops and circles included neutral red segregating spaces (see text-figs. 1–4).   Covell and Scott (1928) gave another explanation, superficially similar, in proposing that chains of neutral red vacuolar spaces formed the basis for an artificial deposition to produce the strings and filaments of the Golgi apparatus. This idea does not appear to have become generally acceptable, because it has been widely recognized that in other cells osmiophile or argentophile material exists apart from vacuolar spaces.   The view put forward by Gatenby did postulate that there was silver or osmic reducing material investing the supposed neutral red vacuoles.

As has been mentioned above, the Italian histologist Ciaccio in 1910 had described various types of sudanophile bodies, fine and coarse, granular and vesicular, in the neurones of frogs, dogs, mice, and other animals.   Two of his figures are given in pl. II, figs. 13 and 14, in the present paper.

In 1944 Baker described the same granules and vesicles in the mesenteric ganglia of the rabbit (our pl. II, fig. 11).   Baker considered that the argentophile net was an artefact, but that the cell contained only vacuoles, a hypothesis along the same lines as Gatenby's 1929 hypothesis (pl. II, fig. 10) ; he also published photographs of the Ciaccio granules, which he considered the true picture of the Golgi apparatus ; that is to say, the Golgi apparatus is a series of sudanophile vesicles more or less joined artificially by the deposition of the silver and osmium of the conventional Golgi apparatus methods.

In 1947 Robert Barer described phase pictures of unstained neurones of a monkey, after fixation in alcohol-acetic acid.   One of his pictures (pl. III, fig. 12, of his paper) shows a white canalicular system, very like the figures we have obtained by phase contrast.   Barer notes that his pictures " show appearances suggestive of Nissl granules, Golgi apparatus, boutons terminaux, and numerous extremely fine cell processes."

Owen Thomas (1948) in his study on the " Spheroid System " of sympathetic neurones has, as we believe, re-described the Ciaccio granules.   We do not propose to give an account of his work here, because we are at present studying the sympathetic neurones in adult animals which show definite stages of cytological senility.   Thomas's work will be reviewed in a forthcoming publication by us.   We can say at present that a perfectly normal Golgi apparatus can be seen in these neurones, and in addition an accumulation of lipid granules as figured by Baker (pl. II, figs. 11 and 12).

In a previous paper Gatenby, Moussa, and Dosekun (1949), taking the axon end of the spinal cord neurone, demonstrated that elongated Golgi filaments were present both after osmium tetroxide and silver nitrate methods, but, most important, could be seen as canals in neurones viewed by the phase microscope of Zernicke.   These authors described as well Ciaccio granules, and mentioned that a more or less intimate connection existed between some of these, or related granules, and the canals of the Golgi apparatus.

We do not propose at present to discuss the literature on Holmgren's canals.
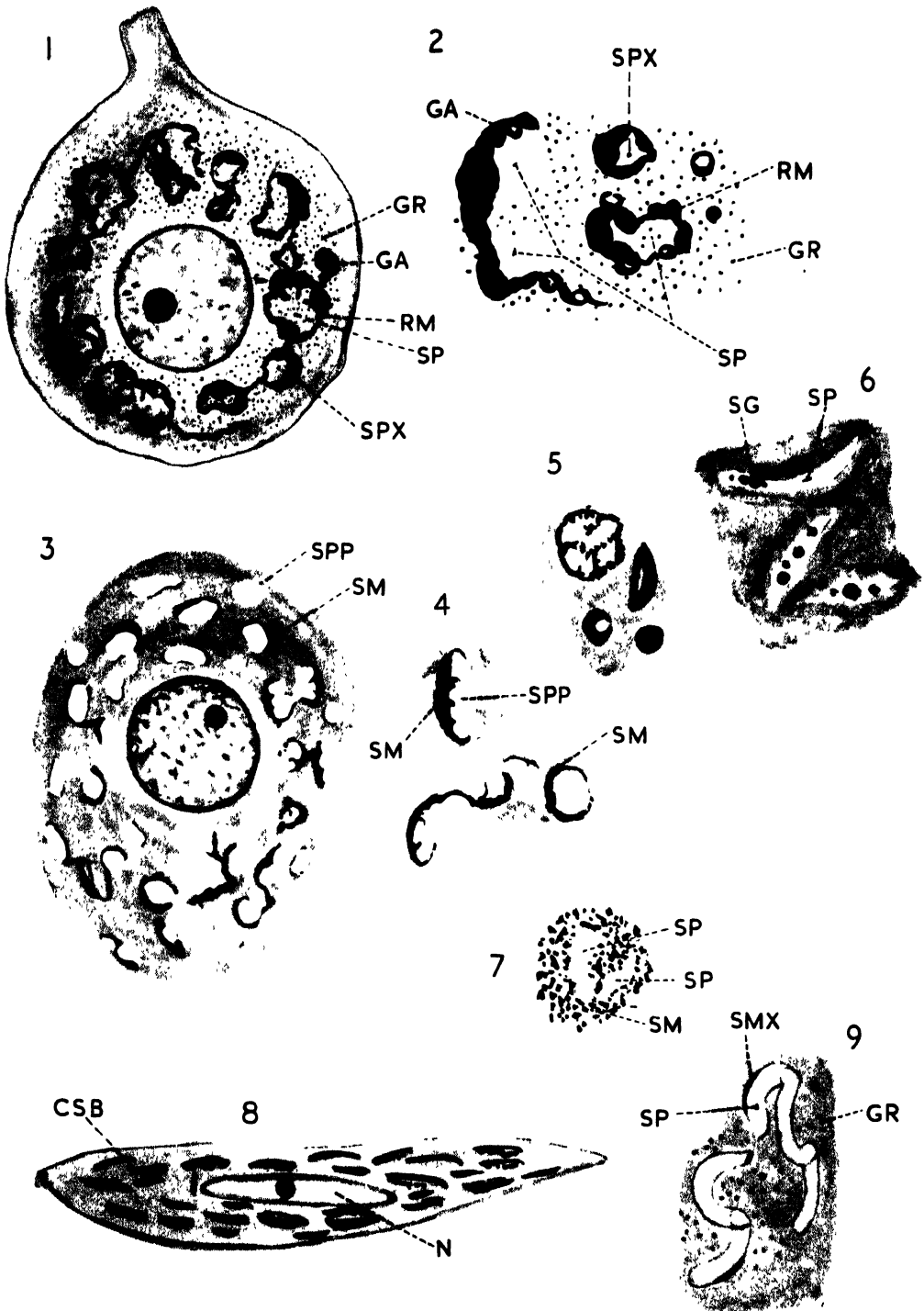
Technique and Material.

The fatty substances were studied after fixation in Aoyama's fluid and staining in Sudan black or red as follows :

Fix in Aoyama's fluid (without subsequent silver) for 3 days, wash in running water for 5–8 hours, transfer to dilute gum overnight, then to thick gum for 8 hours, cut frozen sections and stick them on gelatinized slides ; harden in formalin vapour for 15 minutes, wash in running water for 5 minutes, transfer to 50 p.c. alcohol for 8 minutes, then to 70 p.c. for 1 minute. Stain either in saturated solution of Sudan black B in 70 p.c. alcohol for 10 minutes or, better, in a diluted solution of Sudan black (equal volumes of the above mentioned solution and 70 p.c. alcohol) for several hours. Differentiate in 50 p.c. alcohol for half a minute or more, stop differentiation in distilled water and then mount in Apathy's syrup. In the case of Sudan red, the bottle containing the saturated solution of Sudan IV in 70 p.c. alcohol must be kept at 37° C. ; filter in a clean, hot jar, and then transfer the slide from 50 p.c. alcohol to 70 p.c. alcohol at 37° C. for a minute, and then to the Sudan red jar, where it is left overnight. Differentiate and mount as mentioned above.

The Ciaccio method (formic acid formula) was also employed, but it is inferior to the Aoyama-Sudan black frozen method.

We have found that material soaked and cut in gum is superior to that imbedded in gelatin for 24 hours or cut directly in water.

For the study of the fatty material imbedded in paraffin, the material was fixed in Aoyama's fluid (no silver) for 4 hours, washed in running water for an hour, dehydrated, cleared in xylol, imbedded in paraffin wax and cut into sections of a suitable thickness (5μ). The sections were brought down to 70 p.c. alcohol and then stained in Sudan black B or Sudan IV, as already mentioned for the frozen sections

The Golgi apparatus was demonstrated by the Nassonow modification of Kolatchew's osmic method, as well as by the silver methods of Aoyama and Cajal. The material was all prepared by the junior author. We used also a Cajal section given to one of us by Dr. Wilder G. Penfield, whom we wish to thank.

The General Effect of Sudan Staining.

We found no difference between the results got with Sudan IV and Sudan black B, except in colour. We found that a warm solution of Sudan IV had to be used overnight, and care taken not to over-differentiate in 50 p.c. alcohol. The Sudan black stains very rapidly.

It has long been noted by cytologists of the nervous system that, with silver and osmic methods, the impregnation of two cells side by side may be quite different,[*] even in a preparation which otherwise was considered as perfect as possible. The same thing is shown by the Regaud-Heidenhain method, in which differentiation removes the stain more rapidly from some cells than others. There is considerable variation in the relative cytological ageing of neurones,

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[*] Current text-books of histology describe different sorts of cells in dorsal root ganglia on what we believe to be these cytological variations.

shown by what appears to be a partial dehydration and collapse of neurones here and there in a ganglion. This differential effect increases with age.

Correspondingly, the sudans resemble the osmic methods in revealing such differential effects, and what can be brought out in neurones. Moving the neurones across the field of the microscope, one notes cells with what appears to be the conventional picture of the reticulate Golgi apparatus and cells in which there is no dye, or in which the dye has remained in some other structure. It is this point which we have studied in this paper. We have also noticed how important it is to avoid as far as possible shrinkage of the material by ineffective imbedding in gelatine prior to cutting with a freezing microtome. We wish to emphasize this point. It is a definite pitfall which in future may lead some observers to describe incorrectly the morphology of the cytoplasmic inclusions of these and other cells. It is well known that the neurone is a difficult cell to keep normal in an ordinary histological preparation. It usually lies in a tangle of nerve fibres, from which it is often shrunken away.

Silver Nitrate and Osmic Preparations.

These have been extensively studied in the past, mainly in the form of Cajal or Da Fano (and Kolatchew) preparations. In pl. I, fig. 1, we have drawn a typical D.R.G. neurone. There is a fine granulation (GR) throughout all such cells. These granules are very resistant to bleaching, and have usually been regarded as mitochondria, which we do not now believe to be the case. We consider that the mitochondria of these cells are larger and fewer and can be shown by bleaching out such Kolatchew preparations and staining in Heidenhain. The nature of the fine granulations (GR) is unknown, and will remain so until their origin in embryonic neurones has been carefully investigated.

Turning now to the Golgi apparatus itself, the conventional reticular structure is shown in pl. III, fig. 18. The apparatus, as is well known, varies considerably in texture. In smaller neurones it tends to be coarser and formed of fewer, sometimes very large elements, whereas in larger neurones it is spun out and tends to break up into beaded structures. This applies to both osmium and silver preparations. Now if individual parts of the apparatus, favourable for observation, be studied, it will be noticed that the filaments of the Golgi apparatus often enclose or partly enclose spaces (SP in pl. I, fig. 1). Furthermore in some of these spaces the granulations (GR), already mentioned, pass into the spaces, whereas in other places they do not intrude (pl. I, fig. 2, SP). This appears to show that the cytoplasm just near parts of the Golgi apparatus is different from other regions. In pl. I, figs. 1 and 2, at SPX, spaces free of granulations are depicted. The exact value of this observation is not known to us, but is discussed later.

Now, if such cells as that in pl. I, fig. 1, be changed to the phase microscope, no white spaces are noticeable. In some cells, however, one or two white regions (SPX) may be found in partially bleached osmium, but not in Cajal silver preparations. Now while it is true that many D.R.G. cells apparently show isolated argentophile or osmiophile vesicles, careful focusing usually demonstrates that they are connected more or less completely with other
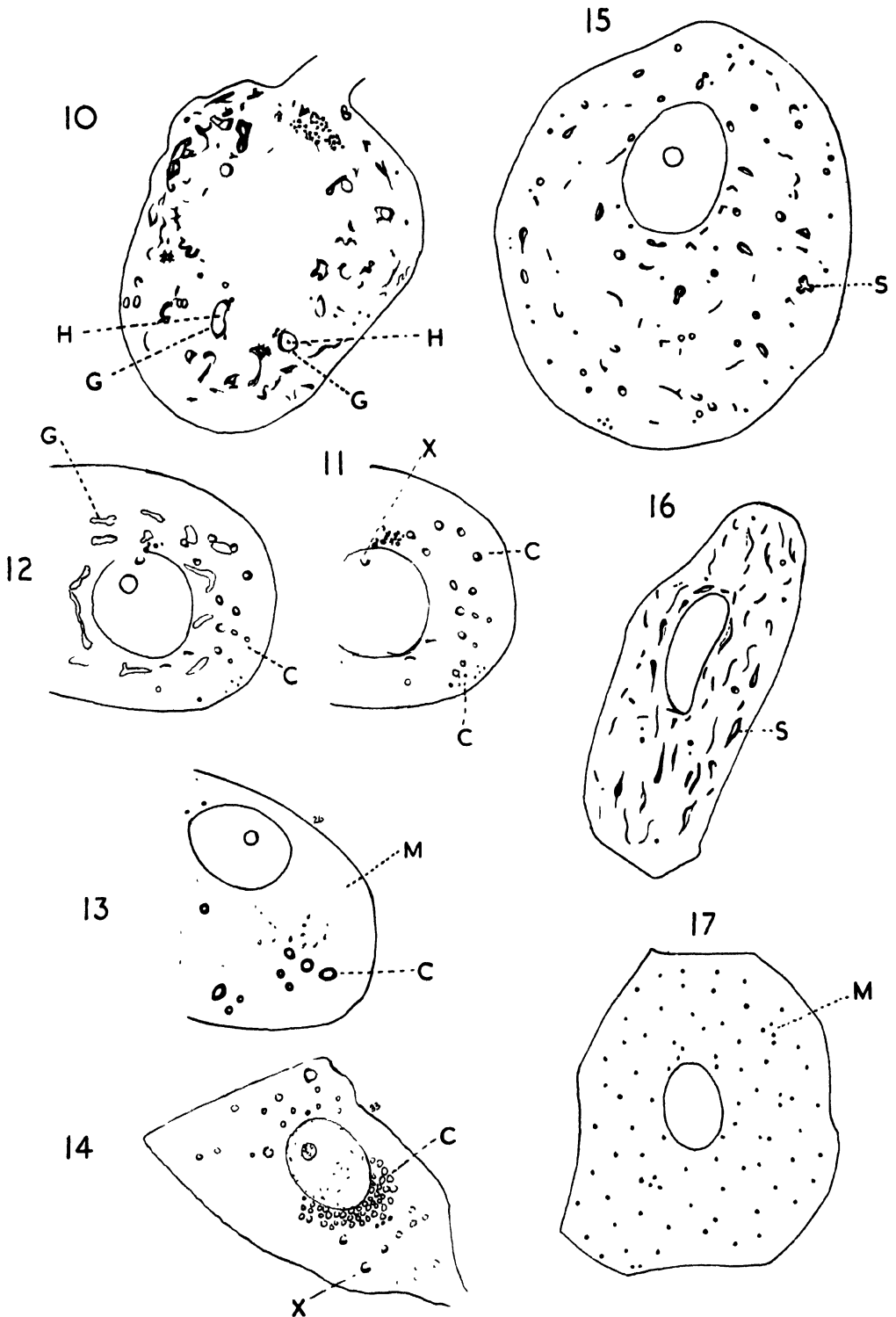
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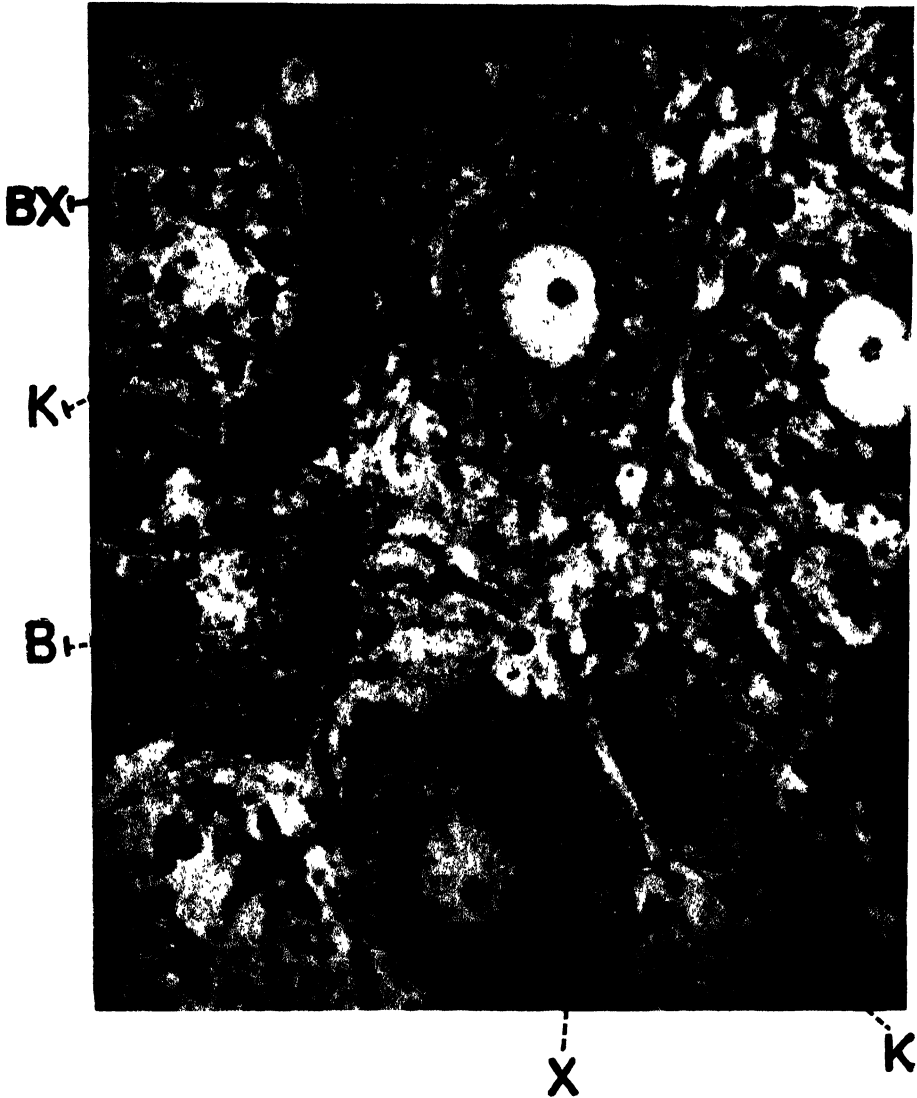
vesicles at a higher or lower focus, or are merely sharply bent loops, cut across so as to produce the effect of rings.

At K, in pl. III, fig. 18, what are apparently cortical canals passing up to pore on the surface are shown clearly. Others can be seen in the photograph. These have been variously interpreted and were regarded as the true Holmgren's canals by Brown (1936).

The Sudan Black Preparation.

Many of our formalin frozen sections stained with Sudan black or Sudan red appear somewhat like fig. 1 on pl. I. Even without the phase microscope, the sudanophile material can be seen apparently to embrace vacuoles. Turning to the phase microscope material one gets the very remarkable appearance, which we have tried to depict correctly in pl. I, fig. 3. Individual parts of such cells are shown in pl. I, figs. 4 and 5. There are white, narrow areas, usually crescentic or ovoid, and capable often of being focused as they pass into upper or lower spaces. That these are not extraphase focal " ghosts " of solid granules is quite clearly and decisively proved by turning back to the ordinary light microscope, where the spaces can still be seen, though less well.

Sudanophile Material in Neurones.

Sudanophile material consists of at the least :

(a) Ciaccio's bodies (" spheroid complexes ") free in cytoplasm (pl. II, figs. 13 and 14).

(b) Flat or scale-like components associated with the Golgi apparatus (pl. II, fig. 15).

(c) Mitochondria (pl. II, fig. 17), after prolonged fixation in formol.

The relationship between (a) and (b) is not clear. In old neurones a mass of sudanophile material in the form of granules can sometimes be seen as well. These are of the Ciaccio type. Category (b) (scale-like components) is left as a small residue in paraffin-imbedded material fixed in formol or formol-chrome, for a few hours up to three or four days ; or post-chromed. The neutral fat is extracted during paraffin-wax imbedding. We have found no way of telling whether this residue is impregnated in silver—that is to say whether the remains of the sudanophile material left in the Aoyama wax section takes part in the formation of the silver or osmic image of the Golgi apparatus.

On the Relationship between the Images drawn in Pl. I, Figs. 1 and 3.

Now what is the true relationship between the silver and osmic net in fig. 1 and the Sudan black or red objects in fig. 3 ? This puzzled us considerably for a long time until we decided to study the 4-hour fixed Aoyama paraffin section (no silver) stained in the Sudan dyes.* It is well known that practically

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* Neglect to make this comparison led to Baker and Thomas making an error.

all work on the Golgi apparatus has been done with paraffin-imbedded sections, and that the impregnated silver or osmic net survives all types of clearing " oils," such as cedarwood oil, xylol, benzol, or chloroform.   Moreover, allowing for shrinkage, the picture given by the Aoyama block cut in paraffin, or by the frozen method is very generally similar.

To our surprise, we found that in material fixed for 4 hours in formol cadmium chloride (Aoyama)—as for this method—brought up through alcohol and xylol, sectioned and stained in the Sudans, showed canals, with here and there shrunken sudanophile beads, shells, or flat bodies, as at SMX in pl. I, fig. 9. These can be seen, with or without staining, with the ordinary and phase microscope.

In paraffin-wax imbedded material prepared by Regaud or Ciaccio (formic acid formula) and Sudan black or red, the flat sudanophile bodies were perhaps clearer, yet not so large as in the Aoyama frozen sections.

We have concluded that the Golgi apparatus in a preparation made with the usual short fixation in formol, then silver, and paraffin imbedding, is the part which remains as white negative images in fig. 3.   Very little of the sudanophile part shown in fig. 3 is left after the paraffin imbedding.

The reticulate effect which the older workers recognized as typical of the Golgi apparatus is produced by silver deposited in and on the large areas which appear as hollow structures under the phase contrast. Since similar reticulate structures undergo definite changes in the cycle of gland cell secretion, as has been shown by many independent studies, we prefer to believe that the sliver or osmic acid is deposited in a definite area.   If the silver and osmic bridges were haphazard and did not differ in coarseness with the type and size of " ghost " under phase, we should be more ready to consider the bridges and filaments to be artefacts.

PHOTOMICROGRAPHS ON PLS. III–VI, FIGS. 18–22.

An attempt has been made to illustrate the above description by photomicrography both with and without phase.   In fig. 18 is a part of the dorsal root ganglion of an old cat, prepared by Dr. Penfield with Cajal's method.   At cell X is a typical coarse-type Golgi apparatus ; at BX, a Golgi apparatus beginning to break down ; and at B, a broken down Golgi apparatus.   In fig. 19 is an Aoyama-fixed (4 hours, no silver) preparation made by the paraffin section method (through absolute alcohol and xylol).   The negative image (ghost) of the Golgi apparatus is marked G.   The same slide is shown under phase in fig. 20.   Compare for size the " holes " in fig. 20, X, with those in fig. 18, X. They are distributed in the same way in both cells.

Now in Aoyama-fixed (no silver) frozen sections (no absolute alcohol or xylol), the sudanophile bodies are shown in fig. 22.   Here and there and especially under reduced light and by focusing, clear canals can be seen (for example outlined in a lower cell in fig. 22).   Turning this preparation on to phase, we find that the apparently solid or curved granules show hollow spaces (G). Most of the hollow spaces (outlined in lower cell in fig. 21) have a sudanophile rim, usually on one side only.   The spaces may appear elongate or spherical,

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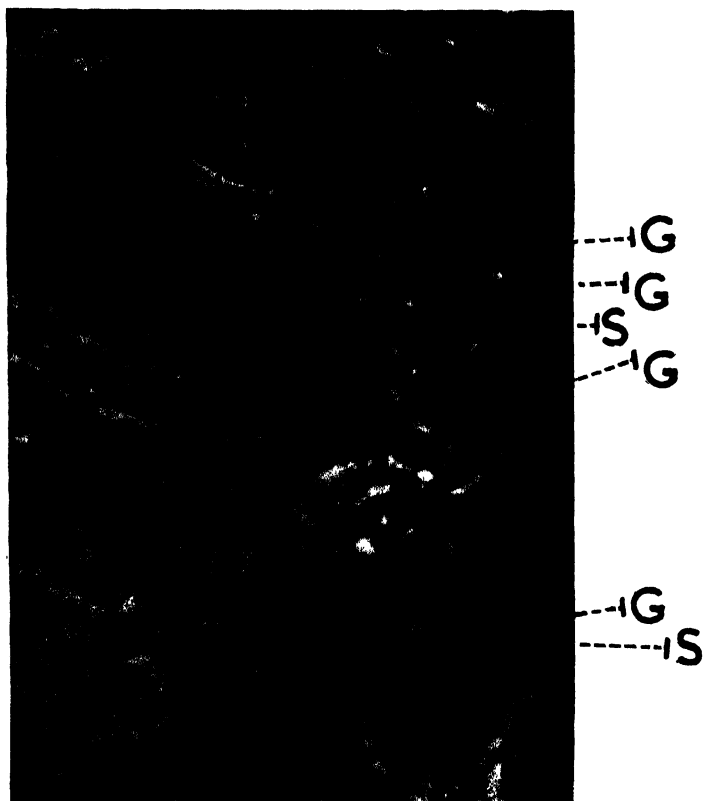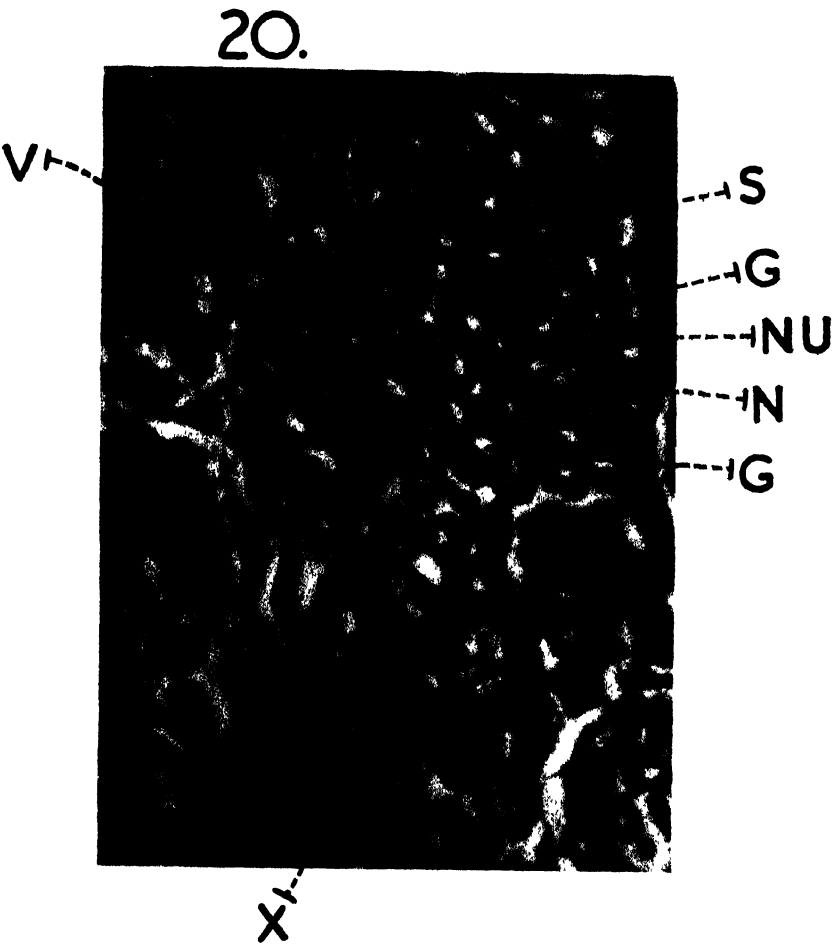
and by focusing they may be seen to run into one another here and there. Now, going back to fig. 19, each space (G) which we consider the Golgi apparatus is accompanied by a dark bead or edge. In fig. 20, V, the canal has a core. We are not able to say whether the core is of the same material and nature as the sudanophile associate on the wall itself. Sudanophile bodies in both positions occur in the same cell side by side.

NOMENCLATURE.

Examination of Baker's plates of photomicrographs of sympathetic ganglia stained by the Sudan black technique and those of Ciaccio shows that the two workers have described the same bodies. It is quite clear that these " spheroid complexes " should be called " Ciaccio bodies " if any name better than " sudanophile granules and vesicles " be needed. The white spaces seen by the phase microscope in any unstained section of a ganglion constitute the Golgi apparatus. This can be best proved by bleaching a silver or osmic Golgi apparatus preparation and comparing the size, shape, and number of the blackened Golgi apparatus and the clear spaces of the bleached cell. What, then, is the status of the sudanophile scale or silver ? We are not tempted to call it a part of the " Golgi complex," " Golgi element," or " Golgi zone " until we know more about other cells. The nerve cell is a very specialized cell. At present we prefer to use the words " Golgi apparatus " for that net-like part which, after 4 hours in formol silvering and paraffin imbedding, remains impregnated in silver. This will avoid confusion and the invention of more new names until the status of the sudanophile associate is cleared up for other types of cells.

HOLMGREN'S CANALS.

Although these so-called canals have been studied considerably, our present knowledge is quite unsatisfactory ; we have made some observations in the course of our work. The only really reliable material for study which we have seen is that cut by the frozen section method (we have not yet tried collodion-imbedded material) ; neither dorsal root ganglia nor the neurones of the spinal cord are satisfactory, only those from sympathetic neurones. The reason for this is that in the two former types of neurones, the Golgi apparatus comes quite close to the cell wall (pl. III, fig. 18, K), and we are also not satisfied that former descriptions of canaliculi opening at the surface of the cell are not in some cases due to cracks caused by paraffin imbedding. The phase microscope has surprised us by revealing the inadequacy of some paraffin sections, in comparison with those made after proper fixation, soaking in gum, and cutting carefully by the frozen method. The mesenteric ganglion cells, as Baker (1944) has stated, appear to have a cortical area free of any kind of Golgi apparatus, and these cells will probably provide the necessary material. We are unwilling at present to offer any further comments. In 1929 the senior writer had vaguely accepted the view that the canals were the vacuoles, or the negative image of a Golgi apparatus, without going into the matter in a critical manner. The phase microscope, with suitable material, should clear up this problem.

It is well to mention that in many epithelial cells the Holmgren's canals are certainly the ghost or negative image of the Golgi apparatus, which in glandular epithelia changes radically during secretion. No question of cortical canals penetrating to the exterior arises in these cases.

How far Cytological Techniques produce Artefacts in Neurones.

Neurones may be fixed in formol, formol-chrome, chrome-osmic, etc., and in every case the phase contrast shows the same spaces which we call the negative image of the Golgi apparatus. Inferior fixation such as alcohol-acetic does not seriously affect the appearance of these spaces. Both silver (Aoyama, Da Fano, Cajal) methods and osmium tetroxide (Kolatchew, Weigl, Sjövall) produce the same sort of Golgi apparatus. In ultra-centrifuging such eggs as those of *Gasterosteus* (Stickleback), the Golgi net may be bent, crushed, distorted and thrown to one side of the cell, but it remains the same always (Singh and Boyle, 1938). What, then, is an artefact? Are the osmiophile or argentophile cords bridging the larger vesicular silvered or osmicated bodies of pl. I, fig. 1 arte-facts? We do not know this for certain. There is no evidence for saying these connecting strands are artefacts. Long cords can be seen in the axon ends of neurones both with silver, osmic, and in unstained sections viewed under phase. The evidence is that long cords actually do exist. Here certainly the hypothesis that the Golgi apparatus is a series of vacuoles is inapplicable.

The slivers and scale-like sudanophile components associated with the Golgi apparatus (pl. I, fig. 9, SMX) are certainly shrunken in the Aoyama wax section preparations. They should be as in pl. I, fig. 4, SM. The conventional formol-silver Golgi apparatus preparation does not therefore give a true picture of the sudanophile associate.

The Significance of the Present Interpretation of the Argentophile and Sudanophile Structures in the Neurone.

Even though we now know this is incorrect, nothing could be more natural than to assume either that the argentophile bodies of the usual silver methods and the sudanophile material are one and the same, or alternatively that the sudanophile material is a " dictyosome " or " lepidosome " edge and the spaces seen with the phase microscope constitute a " vacuome." Should we then assume that the usual Golgi apparatus methods are inadequate? As for showing the sudanophile material, they do not do this at all adequately. The phase microscope does show the shrunken sudanophile sliver or scale as in pl. I, fig. 9, SMX, but we agree that this component associated with the Golgi apparatus of the neurone is, if at all, a very small part of the silvered Golgi apparatus. In a few words, the Golgi apparatus methods show the Golgi apparatus adequately. They show what Golgi, Cajal, and Da Fano considered to be the Golgi apparatus, no more and no less. Is this sudanophile associate of the Golgi apparatus universal in cells? We cannot answer this at present. We can say, however, that the Sudans are not specific stains for any given cell component. We do not know the origin of the sudanophile associate of the

Golgi apparatus. This will need careful examination of developmental stages. Is the sudanophile sliver or scale homologous with the chromophile edge of the invertebrate germ cell dictyosome which also goes red or black in Sudan red or black ? We cannot answer this till more work has been done, but we can say that the neutral fat globule, the dictyosome, the sudanophile sliver, the mitochondria, various yolky bodies, and other fatty materials like the myelin sheath of the nerve all go black in Sudan black. Are these all, then, homologous bodies ? The Sudan dyes have no specificity. The silver and osmic methods for the Golgi apparatus have a much higher order of specificity. So has the Regaud-Heidenhain method for the mitochondria or the Altmann chrome-osmic acid-fuchsin technique. There can be no doubt that the sudanophile material associated with the neurone is not pure fat alone. It has a part which is fixed by formol in 4 hours so as to enable it to survive the hot xylol used in imbedding. It is probably a fatty material in some proteid-like complex. We intend to examine this point later. We can say at present that no fixation accompanied by post-chroming preserves the form and bulk of the sudanophile component through the steps of paraffin imbedding in as successful a manner as is done by the frozen-section technique.

The Chromophobe Part of the Golgi Apparatus.

In Cajal silver preparations we have, in the past, noticed in young and developing neurones that the golden archoplasm or chromophobe area accompanies the parts of the argentophile Golgi apparatus as it breaks up and spreads in the cytoplasm. This golden area seems to disappear as such in the older neurones, but the differences in intrusion of the fine granules (GR) shown in pl. I, fig. 2, would appear to us to indicate that the original archoplasmic area of the embryonic neurone is left in part in the adult neurone. This is meant to say that we think that the original chromophobe area which can be seen in the germ cells, male especially, has existed also in the neurone and probably is found in the adult neurone as a slightly different area of the cytoplasm accompanying the blackened argentophile Golgi filaments. We mention this lest it be assumed that we consider that the two acknowledged parts of the Golgi apparatus, chromophile and chromophobe, are present in the neurone in the form of sudanophile and argentophile areas respectively. We consider at present that the sudanophile area is something new.

The Mesenteric Ganglion Neurones.

On his plate 2, figs. 33–37, Baker (1944) gives some excellent photographs of granular and vesicular sudanophile bodies in the above neurones. These may be compared with Ciaccio's figures. Baker does not state the age of the rabbits he used for making these preparations. In his figs. 34, 35, 36, and 37 the Ciaccio granules he shows are undoubtedly excentric in position. His fig. 41 depicts a typical silver-nitrate Golgi apparatus such as we have also found in the mesenteric ganglion cells. The lipid vesicles of Ciaccio are

definitely, in his figures, included here and there in either osmiophile or argento-
phile strands of the Golgi apparatus. In our material from half-grown rabbit
and the kitten dorsal root ganglion, we never found such markedly excentric and
large lipid bodies, but this marked excentricity of the lipid granules does also
occur in our preparations of the mesenteric ganglia. We believe his material is
from cells which are senile and that all sympathetic ganglia in the adult show
cytological if not functional senility. Baker's fig. 41, by Da Fano's method,
shows a widely spread Golgi apparatus with about fifty possible points or
separate parts of Golgi apparatus which could house or form lipid blebs. His
figs. 33–37 show often less than half such a number, and a quite different distri-
bution. The two things are not the same. The phase microscope settles this
question very efficiently. There are Golgi " vacuoles or canals " under the
phase in all the clear parts of the cytoplasm of Baker's figs. 33–37 corresponding
to neurones which we have studied.

Now as to whether the vesicles depicted by Ciaccio, Baker, and ourselves
segregate neutral red, no one knows. There is no evidence at all. These neutral
red vacuoles might just as well appear in the ground cytoplasm or in the fine
granules (pl. I, fig. 2, GR) described in the present paper. The idea that net
rings or vesicles segregate neutral red (Gatenby, 1929) is just a hypothesis, and
still remains so to-day. We are quite sure in other cells, however, that neutral
red may appear merely in the ground cytoplasm or in prozymogen,
proacrosome, etc., spaces. The phase microscope may be a help on this
question, but no one has used it yet from this aspect so far as we can report here.

We believe that an examination of Dr. Baker's figs. 33–37 and his fig. 41
will go far to convince anyone that he has been dealing with two quite different,
if inter-related, structures.

Discussion.

On the question of rival hypotheses of the nature of the Golgi apparatus, it is
well for the reader to remember that these are based mainly on formalin-fixed
material. It is also to be remembered that every cell with the diverse appear-
ances shown in pls. III–VI has been fixed in the same way.

If the argentophile filaments joining the enlarged parts of the Golgi apparatus
are claimed to be artefacts, so may the Ciaccio granules and enlarged (vacuolar)
parts, as well. Where the Zernicke phase microscope enables one in unstained
cells to observe canals or such bodies as the sudanophile associate materials, the
question of stain artefact, but not of fixation artefact, is cut out. It seems
impossible to believe that bodies in the neurone which can be seen to develop
and spread out during the embryogeny of the cell are artefacts The silver and
osmic methods reveal the Golgi apparatus of the Cavia or insect spermatocyte
in the same way as they do the Golgi apparatus in young neurones and other
cells. But the Golgi apparatus of the spermatocyte can be seen in the living
cell. It seems legitimate to assume that the bodies in gland cells which undergo
phasic changes are not artefacts. This is generally admitted to-day, and before
the introduction of the ultracentrifuge into cytology was one of the main reasons
why it was acknowledged that the silver nitrate and osmic methods do not
produce artefacts when correctly used.
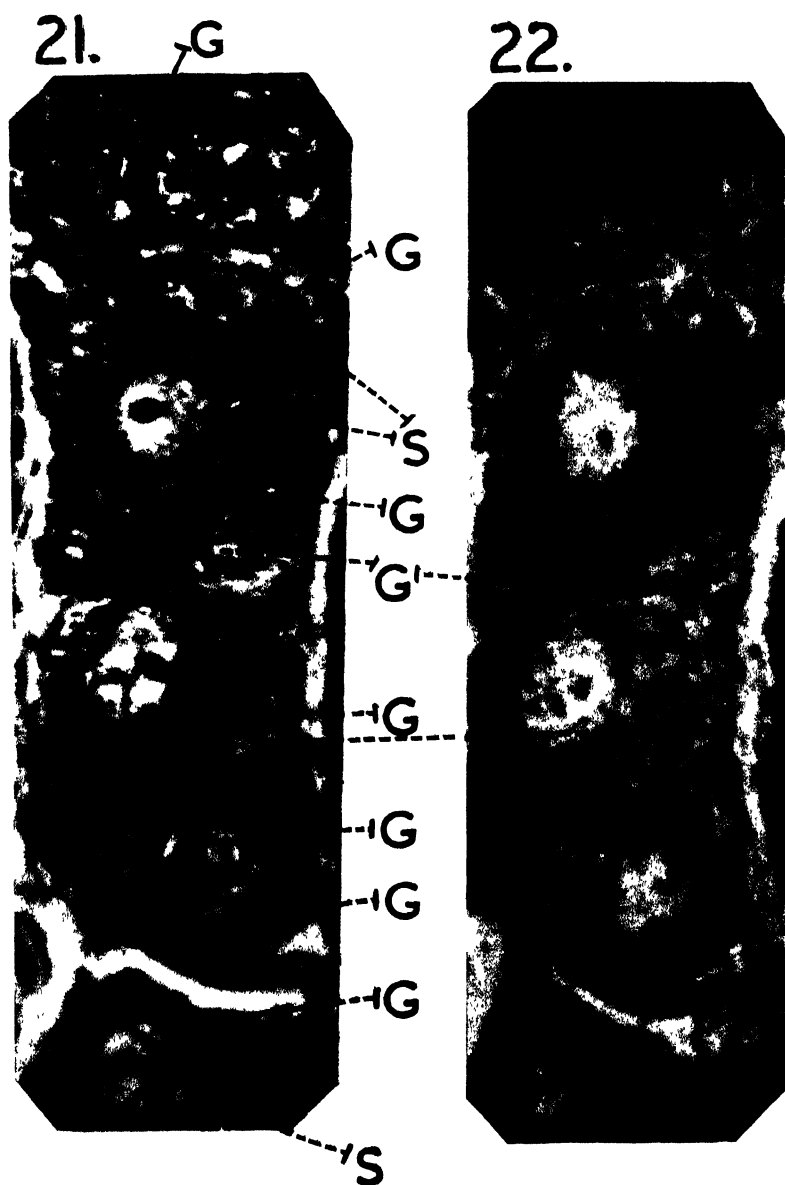
The point of these remarks is that we are just as prepared to believe that Ciaccio's sudanophile bodies or the " spheroid complexes " are artefacts or bubbles produced in the cytoplasm by formalin and Sudan black as we are to believe that the elongate canals which lie alongside them are artefacts too. In the past we have studied the effects of the Janus and other vital dyes on various cells. We have noted that they can be got to stain the mitochondria of cells like the spermatid, and other cells, often with difficulty and by chance, but we are sceptical about the reliability of their use in supra-vital preparations on large inert cells like gland and nerve cells.

There is not the slightest evidence as to what part of the neurone segregates neutral red ; Baker's idea that the sudanophile bodies do so could be correct. The segregates must appear somewhere, and are most likely on *a priori* grounds to appear in the archoplasmic areas beside the Golgi apparatus filaments (pl. I, fig. 2, GR).

No purpose will be achieved at present by attempting to discuss the structure of the cytoplasmic components in the cell in general or the results of the present work. It is obvious that we may be dealing with a specialized formation found in the nerve cell alone. We need to study gland and germ cells as well.

The morphology of ganglion and other cells prepared by silver and osmic methods such as Kolatchew and Weigl has been studied by many authors, and the differences in the appearance of the Golgi apparatus in each type of preparation noted (see for example Dornesco-Busnitza, 1934). What the Sudan techniques will have to add here will doubtless be interesting.

We believe that this present paper emphasizes once again how widely we differ from the vacuole hypothesis of the Golgi apparatus introduced into neurology by Gatenby in 1929 and adopted by Baker in 1944. We consider that Baker's spherical sudanophile bodies are something different from the sudanophile associate of our D.R.G. cell Golgi apparatus, and that had Baker been able to use the phase microscope or had he bleached and stained his Ca–Cd preparations in chlorazol black or Heidenhain, the true Golgi apparatus would have been visible to him apart from his sudanophile granules (" spheroid complexes," Ciaccio granules). We do not think there is a sound foundation for comparing Ciaccio bodies of the Baker type with the sudanophile-associated component of the Golgi apparatus, though, as we have pointed out, until work is done on the development of the neurone, we are on uncertain ground, and these two sudanophile components—granules and associate material—may be phases of the same material. We can put no trust at present in the hypothesis that the sudanophile scale-like component is homologous with the sudanophile edge of the invertebrate dictyosome. We are certain that the Sudans will blanket and saturate many components in the tissues of an animal ; there is no evidence of any special specificity for the Sudans.

Can the phase microscope be said to have shown that the Golgi apparatus of the higher vertebrate neurone is a purely canalicular system, which becomes filled with reduced silver ? Actually it is difficult to understand how the Golgi apparatus spreads out into delicate filaments in what appears to be universally understood to be a highly viscous cell.

We do not know to what extent the silver methods for the Golgi apparatus

are specific. Is the argentophile material in a pancreas acinar cell the same as the argentophile material of the neurone ? Has the latter cell a differently organized Golgi apparatus from the former ? Is the sudanophile material physiologically important in the nerve cell ? Does it respond to fatigue and senility ? Is it merely storage material in the nerve cell alone in this specialized form ? These points we hope to study later.

In view of the results which we have obtained by the Zernicke microscope, some workers would immediately go back to the old idea that the neurone has a canalicular system filled with a material more liquid than the surrounding cytoplasm : the sudanophile associate then becomes a nutrient reserve distributed as necessary, by some means unknown, to all parts of the cell through the canalicular system. Thus fatigue and senility would show not in alterations in the canalicular system, but in the condition of the sudanophile associate. Against this view is our knowledge of the movements, partial disappearance, reappearance, and regrowth of the argentophile material in the secreting cell cycle so familiar to all. But the older canalicular hypothesis is likely to obtain new support from our study of neurones with the phase-contrast microscope of Zernicke.
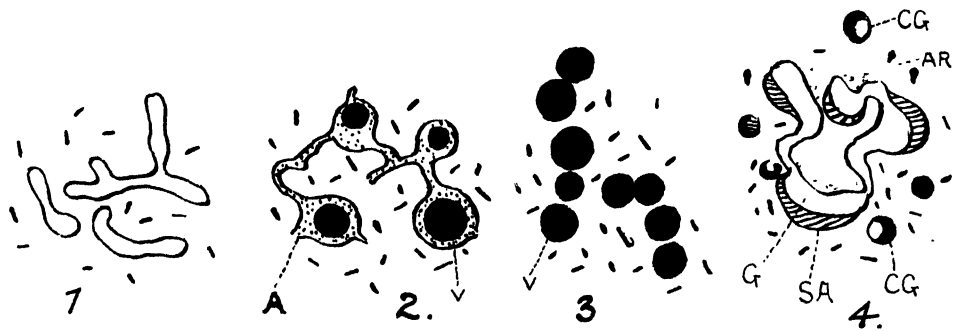
Text-figs. 1–4.

Finally, in text-figs. 1–4, we have given diagrams of the four possible interpretations of the Golgi apparatus. Fig. 1 is the canalicular hypothesis ; fig. 2, Gatenby's 1929 view that the neutral red (Vacuome) segregates appear in the hollow parts of the argentophile material, seen here and there in pl. I, fig. 1, SPX ; pl. III, fig. 18. Text-fig. 3 is the Covell and Scott hypothesis following the original Parat vacuome hypothesis (before Parat introduced the" chondriome actif "), and finally fig. 4 is our present view. The canals (?) are accompanied by sudanophile associates (SA), and in the cytoplasm are free Ciaccio bodies (CG). In all the figures the mitochondria are drawn as short rods. They are usually granules. Fig. 4 refers to the dorsal root ganglion alone.

In the end the controversy on the structure of the vertebrate Golgi apparatus will have to include an explanation of the cortical pores which appear to lead to canals. The original idea of Camillo Golgi postulated that the apparatus was made up of (solid) filaments. Cajal considered the apparatus to be formed of canals, sometimes short or long, sometimes in reticulate form. Holmgren believed that the perinuclear canals shown at K in pl. III, fig. 18, were prolongations of outside trophocytes, and that the canalicular system was a " tropho-

spongium " related to the nutriment of the relatively enormous ganglion cell. It has been suggested that the canals are part of a lymph system. Such workers as Dornesco and Busnitza (1934), believing the osmium tetroxide techniques to be the only reliable ones, considered that a " dictyosome " system was the correct appearance. We have shown here that the Golgi apparatus, as revealed by silver is a deposit, either in or on a canalicular system, to which sudanophile material is in direct relationship. Furthermore that this sudanophile material is almost completely dissolved out by paraffin imbedding, and that it takes little or no part in the argentophile Golgi apparatus of Golgi, Cajal, Da Fano, and others. Moreover, the " spheroid complexes " which were first described and figured by Ciaccio are only doubtfully related to this Golgi apparatus.

General Summary.

1. The Aoyama-fixed frozen section (no silver), stained in Sudan black, usually shows numerous black (or red in Sudan red) vesicles, accompanied by vacuolar spaces : pl. I, figs. 3 and 4. These spaces are very clear under phase. The sudanophile bodies range from loose spaces with sudanophile filaments to solid granules (pl. I, fig. 5).

2. The results of staining in Sudan black tend to differ according to the length of fixation in formol and the method used for producing the frozen sections (pl. II, figs. 15, 16, and 17), whether by gum, gelatine, or water.

3. The sudanophile material can be classified as belonging to five types : (a) sudanophile granules and vesicles free in the cytoplasm (Ciaccio bodies) ; (b) sudanophile scales or leaf-like bodies spread partly around individual parts of the Golgi apparatus ; (c) the fine granulation (pl. I, figs. 1 and 2, GR) ; (d) the mitochondria ; (e) the pigment found in older neurones. Types (a) and (b) may be two forms of the same thing, though there is no good evidence.

4. Sudanophile material is shown in vertebrate neurones by formol fixation and frozen sections or by Ciaccio's post-chroming method, though less well.

5. The Golgi apparatus methods of Cajal, Da Fano, and Aoyama show argentophile material which survives paraffin wax imbedding and the subsequent manipulation of these sections in xylol, and mounting in balsam.

6. Material fixed for 4 hours in formol cadmium chloride (Aoyama), upgraded, and sectioned in paraffin wax, and then stained in Sudan dyes, shows shrunken scales, which are undoubtedly the remnants of the sudanophile bodies mentioned in paragraph 3, (b), in this summary (pl. I, fig. 3).

7. These shrunken scales are not extensive enough to constitute the Golgi apparatus referred to in paragraph 5.

8. The true Golgi apparatus can be seen as large spaces to which the scales (b) are here and there attached (pl. I, fig. 9) or imbedded (pl. I, fig. 6).

9. A possible hypothesis is that the Golgi apparatus of the neurone is a true canalicular system for the distribution throughout the cell of nutriment stored in the sudanophile areas during the histogenesis of the nervous system.

10. The following points are shortly discussed : (a) Whether the Golgi apparatus methods produce artefacts. (b) The relationship between sudanophile and argentophile material. (c) The presence of a granulation in neurones

finer than the mitochondria. (d) The presence of archoplasm (centrosphere or chromophobe material) in the neurone.

SUMMARY BASED ON PLATE.

11. Fig. 18 is a conventional silver nitrate preparation of the Golgi apparatus. The filaments and areas of the latter vary from fine to very coarse (X). If non-silvered cells, fixed for a few hours in formol, are examined with the ordinary microscope, similar empty spaces are seen, corresponding to the size, shape, place, and area of the argentophile Golgi apparatus (pl. IV, fig. 19). These empty spaces have in the past been called " Holmgren's canals." By changing over to the phase microscope, the " canals," and spheres, become very marked, as in fig. 20. Now in the frozen section stained in a Sudan dye, the neurone has many sudanophile granules, fig. 22. Turning to the phase, the same cells show elongate and spherical spaces, often but not always related to sudanophile areas (fig. 21). In Aoyama (formol) fixed material, brought through alcohol and xylol to paraffin wax, sectioned, and then stained in Sudan dyes, a remnant of the sudanophile material is seen (pl. I, fig. 9, SMX).

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DESCRIPTION OF PLATES.

Plate I, Figs. 1–9 ; Plate II, Figs. 10, 15–17 ; Plate III–VI, Figs. 19–22 : Kitten dorsal root ganglion, except Fig. 18 which is from an adult cat (preparation by Dr. W. G. Penfield).

PLATE I.

Fig. 1.—Kolatchew, slightly bleached.
Fig. 2.—Part of same more highly magnified.
Fig. 3.—Under phase, Aoyama Sudan black, frozen section.
Figs. 4, 5.—Parts of same more highly magnified.
Fig. 6.—Aoyama, no silver, paraffin imbedded, Sudan red, showing sudanophile associate (SG) and Golgi spaces (SP).
Fig. 7.—Same method as fig. 3, granular sudanophile substance (SM) and spaces (SP).
Fig. 8.—Same method as fig. 3, gelatine-imbedded frozen section. Shows effects of shrinkage to produce " dictyosomes," which are sudanophile associates.
Fig. 9.—Same technique as fig. 6, but with Sudan black.

PLATE II.

Fig. 10.—Kitten dorsal root ganglion cell after Gatenby (1929), showing a contemporary interpretation of the Golgi apparatus. The argentophile substance (G), or Golgi apparatus, encloses here and there neutral red vacuoles (Vacuome) or Holmgren's canals (H).

Fig. 11.—Mesenteric ganglion cell after Baker (1944) showing Ciaccio (1910) granules ("spheroid complexes"), which Baker believes to be the real Golgi apparatus.

Fig. 12.—Same, with real Golgi apparatus (G) added from preparations by Gatenby and Moussa. See also Baker's fig. 35, bottom left.

Figs. 13, 14.—Ciaccio granules ("spheroid complexes") in vertebrate neurones after Ciaccio (1910). M, probably mitochondria. At X in fig. 14 is the same type of vesicle with the wall on one side thicker than on the other, as pointed out by Baker and reproduced on this plate in fig. 11, X. For the actual Golgi apparatus in Baker's figures, see his fig. 35, bottom left, in a Sudan preparation.

Figs. 15–17.—Three neurones fixed in Aoyama and stained in Sudan black : fig. 17 was fixed several days, figs. 15 and 16 for 4 hours only. Fig. 15 was cut in gum, fig. 16 in gelatine. The granules in fig. 17 (cut in water) are mitochondria, in figs. 15 and 16 they are sudanophile associates and Ciaccio granules.

PLATES III–VI.

Figs. 18–22.—Dorsal root ganglia neurones.

Fig. 18.—Old cat, Penfield Cajal preparation. X, normal ; BX, granulating ; and B, granulated Golgi apparatus. K, cortical canals (Holmgren ?) connecting to Golgi areas.

Fig. 19.—Aoyama fixed (4 hours) paraffin imbedding (through alcohol and xylol) stained section in Sudan black. Golgi apparatus (G) has granule (S) in most of the canals. This is regarded as the shrunken sudanophile associate.

Fig. 20.—Same cell. Compare cell X and NU, with cell X in fig. 18 for size and shape, etc., of Golgi "ghosts" (G).

Figs 21, 22.—Same cells under phase (fig. 21) and ordinary microscope (fig. 22). The cell at the bottom of fig. 22 has the Golgi apparatus lined in. See spaces in cell above. The left is under the phase, showing canals more clearly. It should be noted that at varying focuses this preparation showed vacuoles running one into another. It is much thicker than the other sections in figs. 18–20, and so is not a good object for comparison with 5μ paraffin sections.

535.824 XVIII.—OBSERVATIONS ON CONDENSERS, OBJECTIVES AND EYE-PIECES.

I. MICROSCOPE CONDENSING SYSTEMS.

By B. O. PAYNE, M.Sc.

ONE PLATE AND TWENTY-FOUR TEXT-FIGURES.

THE ensuing paper is one of a series intended to enable microscope users to keep abreast with modern practice in this field. It will be found that most of the apparatus described is available from a number of manufacturers both in this country and abroad, but where an accessory is a speciality of one maker only, this fact is usually referred to in the text.

TRANSMITTED LIGHT SUBSTAGE CONDENSERS.

Primary Considerations.

In considering the characteristics of practically any optical system there are two sets of planes which are of great importance. Firstly, those conjugate to the plane of the object itself, and secondly, those which are conjugate to the exit-pupil of the system. In the case of the compound microscope the exit-pupil is usually an image of the aperture of one of the lenses of the objective, and its diameter is limited by the size of the latter. The field of view is normally restricted by the image of the aperture of a stop in the focal plane of the eyepiece and it is the primary function of the substage condenser to ensure that the whole of the field of view can be illuminated evenly by cones of rays of sufficient angle to fill the back aperture of the objective with light. In order to accomplish this it should be capable of forming an image of the source or of the illuminated field iris substantially free from spherical and chromatic aberration in the plane of the object.

In addition, in order to avoid glare and to give added flexibility, it is desirable to be able to control the illumination by means of iris diaphragms, one—the substage iris—may be placed immediately below the condenser, controlling the aperture of the illuminating pencils ; the other—the field iris—being in a plane conjugate to the object and often mounted just in front of the source of illumination.

Fig. 1 shows a simple lay-out for the optical train of a microscope as commonly used for visual observations, while fig. 2 shows an arrangement for photomicrography, with Kohler illumination. In each case the sets of conjugate planes are marked AA' and BB', etc.

An optically satisfactory, but costly and inconvenient, method of substage
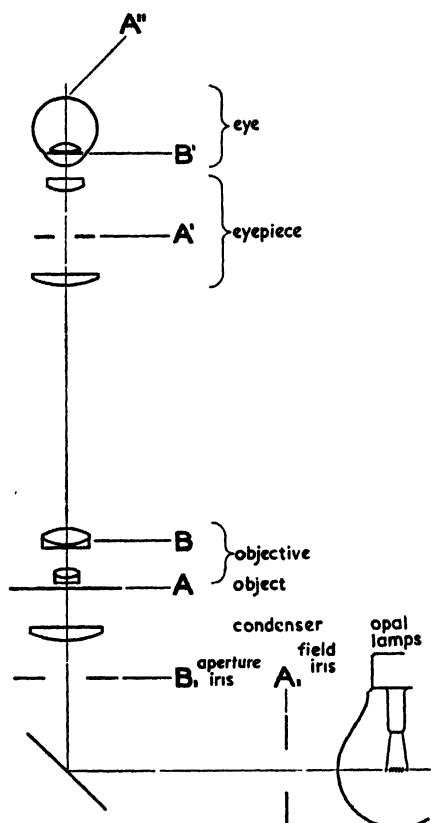
Fig. 1.—Schematic lay-out of micro-
scope system for visual observation.
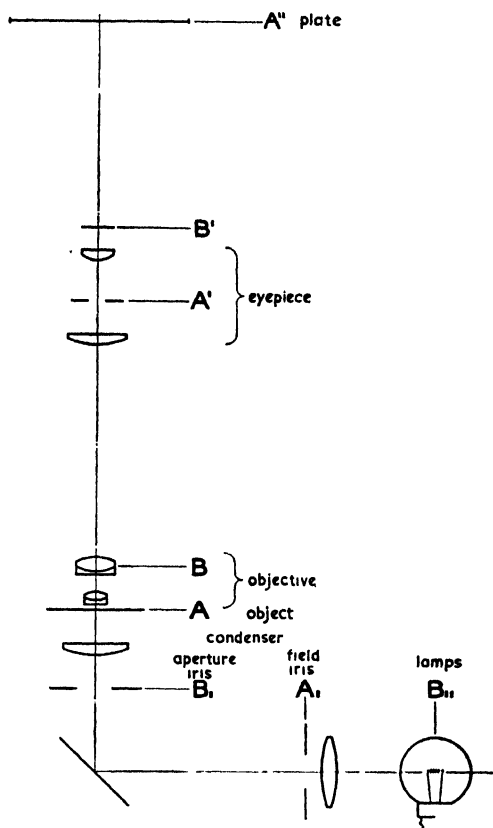
Fig. 2.—Schematic lay-out of microscope
system set up for photomicrography
with Kohler illumination.

illumination would be to employ another identical objective as condenser with
each objective used. It is fortunate that the coherency requirements do not
necessitate as perfect a correction in the substage condenser as in the objective
itself, thus enabling a compromise to be effected so that one condenser is able
satisfactorily to illuminate the 0·1 inch diameter field of a 16-mm. 0·28 N.A.
objective and the 0·01 inch diameter field of a 1·8 mm. oil-immersion lens.

Most of the desirable and undesirable features of the substage condenser can
be illustrated by describing the construction of some of the more usual types.

The Simple Abbe Condenser is shown in fig. 3 and is the commonest and most
long-suffering of all. It is, in fact, to be deplored that the name of Abbe has
become linked with an accessory of such relatively poor performance, since even
at the time of its introduction much better achromatic condensers were obtain-
able. Its chief merits are its cheapness, long working distance, and adaptability,
but it is non-achromatic and suffers from severe spherical aberration.

The effect of the latter will be appreciated from a study of fig. 4, in which
the paths of rays from a point in the plane of the field iris are depicted near the
plane of the object. It is evident that the working clearance decreases rapidly
with increasing numerical aperture, and it is therefore often necessary to raise
the condenser when changing from a low- to a high-power objective. Even

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when this has been done it may be impossible to fill the complete aperture of the objective unless the lamp iris is opened up to a greater diameter than is necessary to illuminate the field of view.   The above defects are aggravated by the chromatic aberration of the condenser.

Fig. 3. The Abbe condenser.
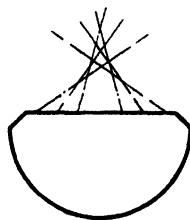
Fig. 4.—Paths of rays emerging from the Abbe condenser, illustrating the effects of spherical aberration.

Pl. I, fig. 1, shows the pencils of rays issuing from an Abbe condenser, photographed by means of the fluorescence excited by the rays in a block of uranium glass.   Pl. I, fig. 2, shows the rays from the same condenser, but with the aperture iris reduced in diameter.   It is evident that although the numerical aperture of such a condenser may be stated to be 1·2, its aplanatic cone will have a very small angle and little is to be gained by oiling its front lens to the slide.

The Three-lens Abbe Condenser is illustrated in fig. 5 and has a somewhat
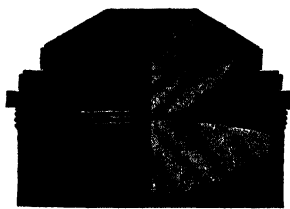
Fig. 5.—The three-lens Abbe condenser.

better spherical correction than the simple Abbe.   It is often rated at 1·4 N.A., but suffers from severe spherical aberration at this aperture and is uncorrected for colour.   The ray pencils shown in pl. I, figs. 3 and 4, make it evident that the aplanatic cone is quite narrow and that the working distance decreases with increasing numerical aperture.   Despite its limitations, this is a useful condenser for much routine work, especially where the utmost resolution is not required from the oil immersion objectives, but it should not be employed when the best possible performance is required from an objective of greater numerical aperture than about 0·6.

The aberrations of an Abbe condenser can be demonstrated in the following manner.   A transparent object is set up on the stage and examined with an objective of fairly high power, say a 4-mm. of about 0·85 N.A.   The lamp iris is stopped down to a minimum with the condenser iris fully open, and the back lens of the objective is examined with the eye-piece removed.   On racking the condenser up it will be found that it is impossible to illuminate the whole back aperture of the objective.   At best only a portion of the full aperture is covered and hence it is impossible for the objective to work at full efficiency.   In addition,
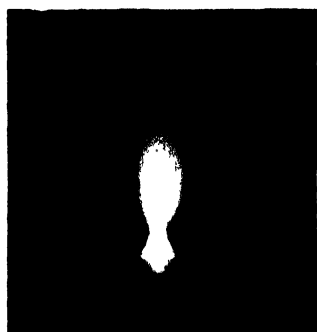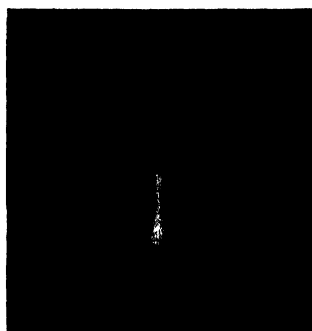
Plate I. Fig 2

Plate I. Fig 1
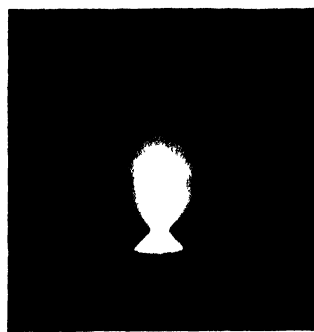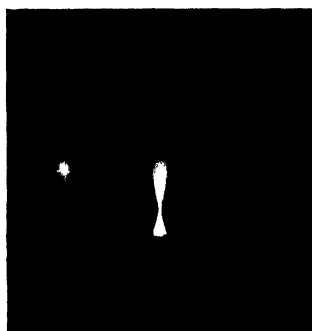
Plate I. Fig 3

Plate I. Fig 5
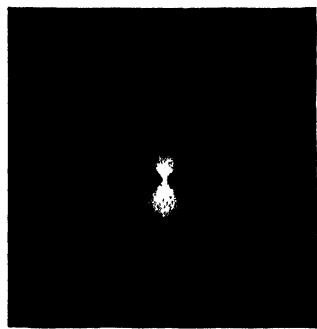
Plate I. Fig 4
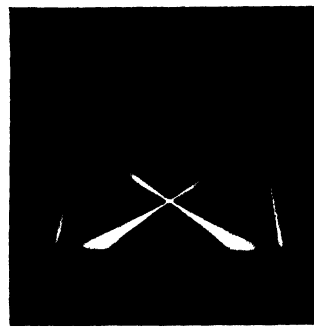
Plate I. Fig 6
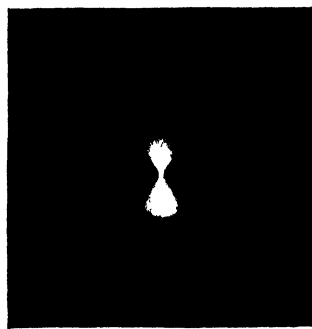
Plate I. Fig 7

the edges of the illuminated zones will be observed to be coloured owing to the lack of achromatism of this condenser. These effects should be compared with those which are obtained when a well corrected achromatic condenser is tested in the same manner.

Three-lens " Aplanatic " condensers are available in which a somewhat improved spherical correction has been obtained by the use of special glasses, but even so considerable spherical and chromatic defects remain.

Achromatic Condensers.—A greatly improved spherical correction is made possible by achromatizing the condenser. Where exacting work has to be carried out with high-power objectives, an achromatic condenser should always be employed, although it should be noted that it is the improved spherical correction obtainable which is of greatest importance. Achromatization gives a better image of the field iris in white light, but has little effect on the resolving power. These condensers are made in various types, two of which are shown in figs. 6 and 7. An aplanatic cone can be obtained up to an N.A. of over 1·3.

Fig. 6.—Universal 1·0 N.A.
achromatic condenser.

Fig. 7.—Achromatic oil-immersion
condenser 1·3 N.A.

The type shown in fig. 6, having an N.A. of 1·0, is convenient for many purposes, has a somewhat longer focal length, and, of course, need not be oiled to the slide. It is evident from pl. I, figs. 5 and 6, that all rays from the centre of the lamp iris are brought to a common focus and that the aplanatic cone includes the full aperture of the condenser.

The working clearance of achromatic oil-immersion condensers is not great, being usually only just sufficient to illuminate through a standard 1·2 mm. thick slide. This renders them unsuitable for some types of low-power work where a longer clearance may be desired. In some cases it is possible to unscrew the front lens, leaving behind a well-corrected system of lower numerical aperture, but having an increased clearance and longer focal length, enabling a larger field of view to be illuminated.

Achromatic and aplanatic condensers are usually corrected to give their best performance with the lamp iris arranged at a specified distance, such as 10 inches, away from the back lens. If this distance is varied then the corrections will suffer in much the same way as those of an objective with varying tube-length. This may also happen if the lamp is brought nearer or a negative spectacle lens is introduced in order to give increased working clearance.

The Focal Length of the Condenser.—Manufacturers do not, in general, offer a range of condensers of varying focal lengths, although there is often a choice of two focal lengths in condensers of a given type, and it is therefore desirable

to appreciate the effect of this factor upon performance. If we assume that the condenser is set up to form an image of the lamp iris at a standard distance, then the diameter of the area illuminated in the plane of the object will be roughly proportional to the focal length of the condenser. Hence, it will be of advantage when low-power objectives are to be used to work with as long a focal length of condenser as possible. This may sometimes be achieved by removal of one or more lenses from the front of the condenser, which then works at reduced aperture. On the other hand, the diameter of the back aperture of the condenser is also proportional to the focal length for a given numerical aperture, and this is often limited by mechanical considerations. If Kohler illumination is to be used it is important that the image of the source of light formed on the back of the condenser should be large enough to give a cone of the required numerical aperture, and hence for high-power work a fairly short focal length is often preferred. These factors must be balanced one against the other in choosing a condenser for any given purpose.

Centring of the Substage Condenser.—It is desirable to be able to centre the condenser so as to bring it in line with the optic axis of the objective and eye-piece, but on the cheaper microscopes this adjustment is done once and for all by the maker and the user is unable to alter it.

It is nowadays usual practice in the more expensive instruments to provide centring screws by means of which the substage condenser and iris may be moved together. The iris is not usually independently adjustable, but in some instruments it can be moved in a lateral slide and thus produce oblique illumination if desired.

Pancratic Condensers.—There has been no radical alteration in the design of the simple substage condenser over more than forty years, but recently pancratic systems have been designed with the object of enabling the change from a small illuminated field of view at large numerical aperture to a large illuminated field at small numerical aperture to be made in the simplest possible manner. Such arrangements are also more economical in their use of the light source than the usual fixed systems in which much of the light collected by the lamp condenser is wasted when low powers are used.

The lay-out of the Zeiss system (British Patent No. 483,633) is shown diagrammatically in fig. 8.

The whole unit complete with lamp can be fitted into the substage in place of the normal condenser. The numerical aperture of the illuminating beam
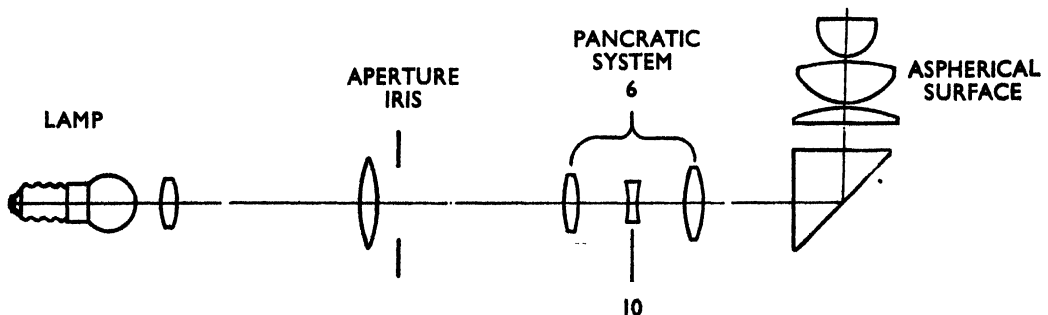
Fig. 8.—The Zeiss pancratic condensing system.

can be varied between 0·16 and 1·40 by means of an engraved rotating sleeve which moves lenses 6 in relation to lens 10. The system employs a small 4-watt bulb and may also be used for dark-field illumination up to a numerical aperture of 0·65.

It should be noted that an aspherical surface is employed in order to improve the correction for spherical aberration.

Other systems have been described by Bausch and Lomb (Benford 1947), who also offer a simpler arrangement in which the lower component of the substage condenser is movable in order to vary the focus.

Condensers for Polarizing Microscopes.—When a calcite prism is employed as polarizer the diameter of the condenser is severely restricted by the aperture of the prism. Hence, if the numerical aperture is to be made large enough to be suitable for conoscopic observations a condenser of very short focal length is necessary. This necessitates the removal of a lens when it is required to illuminate a large field at low aperture for observations in " parallel light." Recent improvements in the quality of polarizing films have made them suitable for use in place of nicol prisms, thus enabling the back aperture and focal length of the condenser to be increased. The same condensing system can then be used for both types of observation, only the setting of the substage iris being altered (Hallimond and Taylor 1946).

DARK-GROUND ILLUMINATION OF TRANSPARENT OBJECTS.

Patch Stops.

No special condensers are needed in order to obtain dark-ground illumination with low- or medium-power objectives. All that is necessary is to mount annular stops (fig. 9) behind the normal substage condenser. It is best to have a stop for each objective of such a diameter that the direct rays are just excluded over
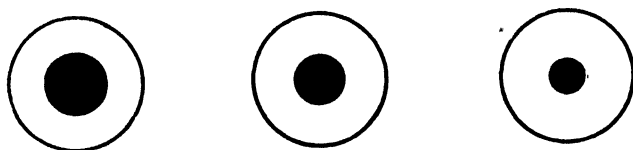
Fig. 9.—Set of patch stops for use with low- and medium-power objectives.

the desired field of view. Sets of such stops are obtainable from most manufacturers and can often be inserted in the filter mount below the condenser. Similar stops are available for producing oblique illumination.

When using patch stops with condensers having numerical apertures greater than unity, it is of advantage to oil the slide to the condenser. This enables the object to be illuminated by rays which would otherwise be totally reflected. With a well corrected condenser quite good dark ground results can be obtained with objectives up to about 0·8 N.A. although the illumination will be less intense than is obtained with a catoptric system. For low powers it is possible to remove the condenser front lens in order to illuminate a greater field of view.

It should be noted that in order to produce dark-ground illumination over

an extended field of view, the minimum numerical aperture of the illuminating beam must be greater than that of the objective in use. If the two are equal, dark-ground effects are produced only at the centre of the field. The reason for this will be apparent from fig. 10.
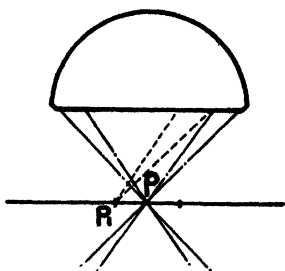
Fig. 10.—Dark-ground illumination over an extended field of view.

Fig. 11.—Dark-ground condenser (fixed focus).

For the central point P in the object field none of the illuminating rays can pass through the objective, but it is evident that for a point P, away from the centre there may be rays which can gain admission and ruin the dark-ground effects.

Catoptric Illuminators.

In order to obtain brilliant dark-ground effects at high powers the maximum aperture of the illuminating beam should be as great as possible and for this reason the illuminator is usually oiled to the slide. In many catoptric dark-ground illuminators the minimum numerical aperture of the illuminating pencils is in excess of unity and such condensers are therefore useless if not immersed.

One of the most useful dark-ground illuminators for general purposes embodies two spherical reflecting surfaces and can be obtained either with fixed focus (as shown in fig. 11) or with the focus adjustable for various slide thicknesses (fig. 12).* When used in conjunction with objectives of 1·3 N.A. and over it is necessary for the aperture of the objective to be cut down somewhat by the use of an internal stop or of a funnel stop mounted just behind the objective lenses. It is also possible to obtain objectives incorporating an iris diaphragm, but unless this is mounted between the objective components or very close to the back lens it is necessary to reduce the numerical aperture rather more than would otherwise be the case in order to obtain a satisfactory dark-ground image.

Condensers of the above type suffer from a certain amount of spherical aberration (see pl. I, fig. 7). A more perfect spherical correction is obtained by the use of the cardioid condenser.

This condenser, as made by Zeiss, consists of two elements arranged so that the ray paths are as shown in fig. 13.

The underlying theory can be explained with the aid of fig. 14.

Let the curve OPQ represent the cardioid $r = 2a(1 + \cos \theta)$ having its origin at the point O and consider a ray from O, making an angle θ with the

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* This type was first manufactured by R. & J. Beck.
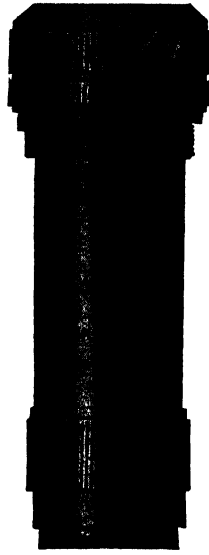
Fig. 12. Dark-ground illuminator, adjustable for various slide thicknesses.

axis OQ, intersecting the curve and being reflected at P. If the ray makes an angle i with the normal at P then it is easy to show that

$$\tan i = \frac{1}{r} \cdot \frac{dr}{d\theta} = \frac{1}{r} \cdot - 2a \sin \theta = \frac{- \sin \theta}{1 + \cos \theta} = - \tan \frac{\theta}{2}.$$

Hence, the angle of incidence is such that the reflected ray PT makes an angle θ with the incident ray OP.

If now, from a point S on OQ, distant a from O, we draw ST parallel to OP to intersect the reflected ray at T and construct a circle of radius ST and centre S, it is evident from the geometry of the figure that the ray PT will be reflected from this circle in a direction TR parallel to the axis OQ.

Now ST $= $ OP $-$ OS cos θ $-$ PT cos θ
    $= r - 2a \cos \theta$
    $= 2a$ and is therefore independent of θ.

Hence, all rays passing through O and being reflected from the cardioid and circle will emerge parallel to the axis and the system is free from spherical aberration.
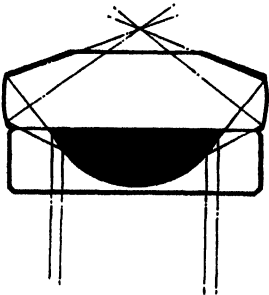
Fig. 13.—Cardioid dark-ground condenser.
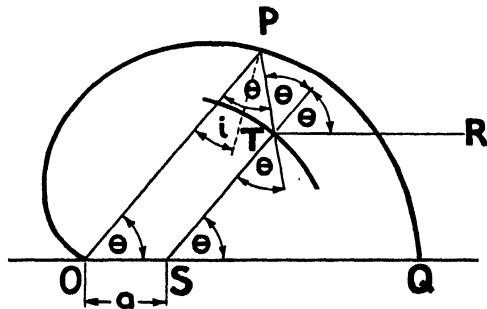
Fig. 14.—Theory of the cardioid condenser.

In practice it is usual to represent the cardioid, of which only a narrow portion is required, by a zone of a circular surface of revolution which most nearly approximates to the desired shape.

Another form of dark-ground illuminator which is of interest in that it has been designed to enable immersion objectives up to 1·40 N.A. to be used at full aperture is the Nelson Cassegrain type, made by Watson. This is shown in fig. 15 and embodies an arrangement of spherical surfaces similar to that found

A. Quartz condenser lenses.
B. Quartz cone.
C. Conical ring reflector.
D. Quartz front lens.

Fig. 15.—Nelson Cassegrain
dark-ground.
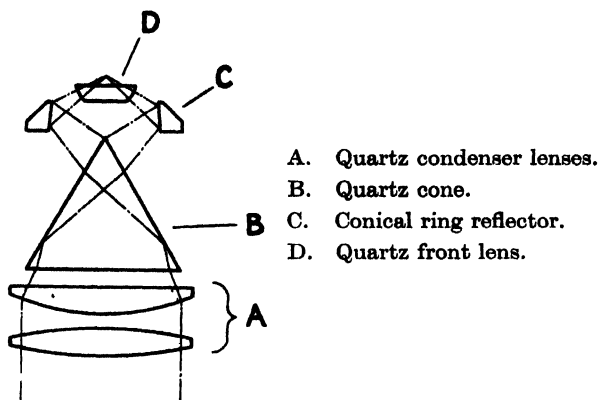
Fig. 16.—Dark-ground illuminator for ultra-violet
microscopy.

in the Cassegrain reflecting telescope. It should be remembered that such condensers can function satisfactorily only if the object is mounted in a medium of refractive index higher than the maximum numerical aperture given by the illuminating beam. It would therefore not be possible to use the above type for the examination of objects in an aqueous medium without some reduction in the objective aperture.

For dark-ground illumination with the ultra-violet microscope where a high intensity of illumination is of great importance, the loss of the central portion of the illuminating beam in the conventional type of condenser is a serious matter. To overcome this difficulty, cone-type illuminators have been designed (Barnard and Welch 1936).* A recent form employing only spherical and conical surfaces is shown in fig. 16. These systems have the further advantage that an iris diaphragm can be fitted which, when closed, increases the minimum numerical aperture of the illuminating pencils. They may also have applications in visible light microscopy.

" Optical Staining " Methods.

It is sometimes of advantage to superimpose dark- and bright-ground images of the same object in different colours in the same field of view. This was achieved in the Zeiss " Micropolychromar " system by the use of annular colour filters mounted in a special substage unit (British Patent No. 360,782).

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* First described SMILES, J. (1933)—" Dark-Ground Illumination in Ultra-Violet Microscopy." J. Roy. Micr. Soc. **53**, 203.

More recently, Merton (1949) has suggested a method in which a blue darkground image is viewed against a background illuminated in its complementary colour (deep yellow). The yellow filter, in the form of a circular disc of just sufficient diameter to illuminate the full numerical aperture of the objective, is surrounded by a clear annulus. The intensity of the bright field is varied by the use of an adjustable wedge-shaped blue filter mounted in front of the lamp. By varying the density of the lamp filter a variation from pure dark-ground to almost pure bright field effects may be obtained.

ILLUMINATION OF OPAQUE OBJECTS.

[*Note.*—Since it is the purpose of this paper to deal specifically with condensers as opposed to methods of illumination generally, we shall not consider such illuminating systems, as are used for macro work, in which no special condensing system need be incorporated.]

The bright-field illumination of opaque objects is usually accomplished by employing the objective as its own condenser. This is done either by mounting a partially reflecting surface between the objective and the eye-piece so that light can be projected through the objective on to the specimen or by employing a totally reflecting surface (such as a prism) immediately behind the objective to illuminate through a portion of the back aperture, leaving the remainder free for the passage of the image-forming light.

Normal Incident Illumination.

This is often achieved by mounting a thin glass reflector at 45° to the optic axis of the instrument, as in fig. 17, and introducing the light from the side in a horizontal direction. More usually the slightly more complex arrangement of
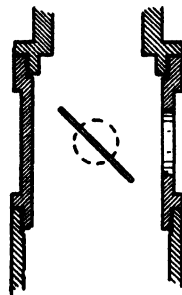
Fig. 17.—Simple cover-glass illuminator.

fig. 18 is used. The area of the field illuminated is controlled by the aperture of the iris diaphragm (*f*). The lens (*e*) is positioned so that it forms an image of the aperture iris (*p*) near the back focal plane of the objective and at the same time forms a virtual image of the field iris in a plane corresponding with the eyepiece focal plane. If the object is flat, highly polished and mounted with its face perpendicular to the optic axis, an image of the aperture iris is formed at the back of the objective by light which has traversed the latter and been
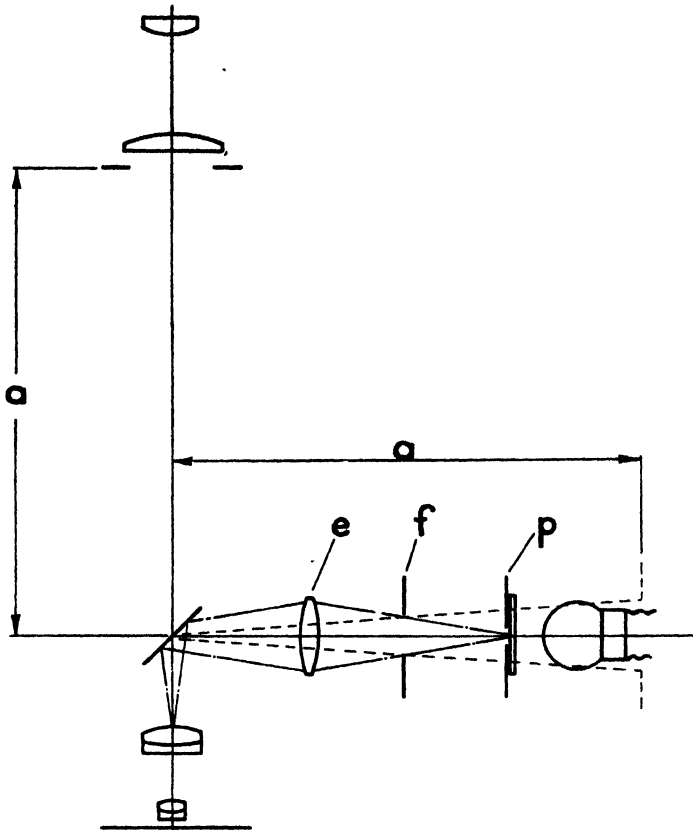
Fig. 18.—Normal incident illumination.

reflected back from the object. By tilting the object this image is displaced and a rather unsatisfactory form of oblique illumination can be obtained. Units of the above type can be obtained with built-in illumination or for use with an external source (fig. 19).

A considerable light loss is inevitable with such a system, for if, say, 10 p.c. of the incident light is reflected from the surfaces of the reflector (which must be thin in order to avoid double images) this will be reduced considerably by double

Fig. 19.—Illuminator unit for normal incident illumination.

transmission through the objective and at most only about 5 p.c. may be available in the image plane. These losses can be reduced to a minimum by coating the reflector surfaces with a high efficiency film and by coating the objective surfaces with anti-reflecting films. As much as 20 p.c. of the incident light can then be made available in the final image plane.

It is important to coat the lenses of objectives for use with incident illumination systems in order to reduce glare in the field of view due to back reflection of light from the objective surfaces, which, in the case of an object of low reflectivity, can easily exceed in intensity the image-forming light.

The Polarizing Vertical Illuminator.

An ingenious method of increasing the illumination of such systems has been described by Foster (1938) and marketed by Bausch and Lomb. The principle is illustrated in fig. 20. The two sections A and B of a calcite prism are cemented
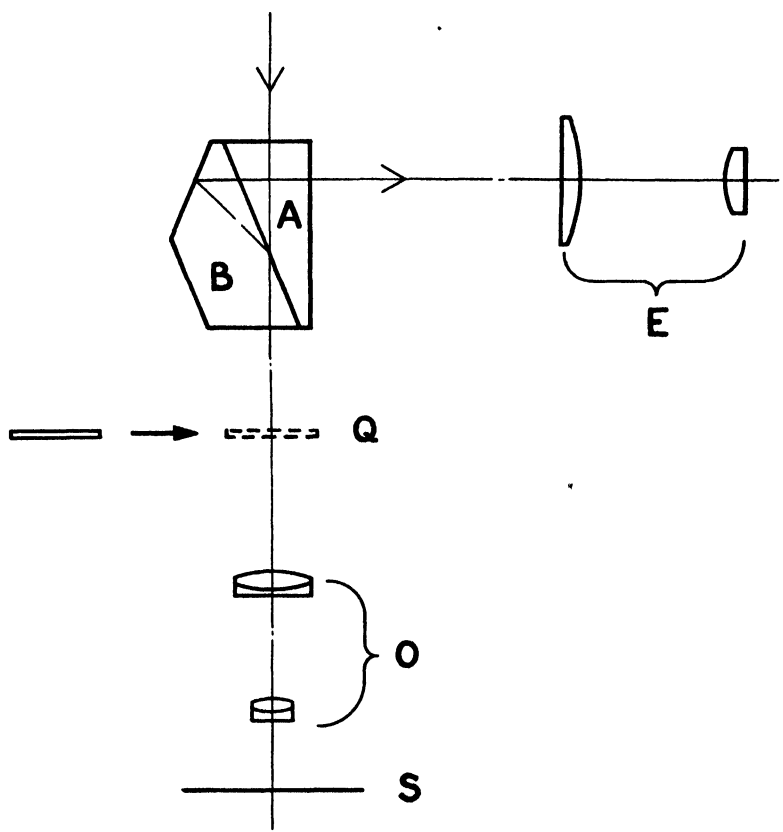
Fig. 20.—The polarizing vertical illuminator.

together with a material having a refractive index which is such that when light from the source meets the interface the ordinary ray is reflected and the extraordinary ray is transmitted unchanged to the specimen. A perfectly reflecting specimen would then return the light with its plane of polarization

unaltered. It would therefore be transmitted back to the source and no light would reach the eye-piece E. In other words, we have effects similar to those obtained in the polarizing microscope with crossed analyser and polarizer.

If, however, a suitable orientated quarter-wave retardation plate Q is introduced between the prism and objective O, this rotates the plane of polarization of the returning light by 90°. (*Note.*—Owing to double transmission the quarter-wave plate introduces a half-wave retardation between the two rays.) What was the extraordinary ray now becomes the ordinary ray on its return through the prism and is therefore totally reflected. The eye-piece is then illuminated by an intensity amounting to nearly half that of the incident light. By the introduction of a half-wave plate in place of the quarter-wave plate sensitive tint effects are obtained.

It is perhaps a disadvantage that with the above system polarized light must be used, whether required or not, for normal incident illumination, and there is some loss of flexibility when the apparatus is used as a polarizing microscope, since it is impossible simply to rotate the polarizer and analyser vibration planes relative to one another by any desired amount.

The Prism Illuminator.

In this type of vertical illuminator a small prism or metallic reflector is mounted just behind the objective and set at 45° to the microscope axis so that light can be directed from the source through the objective on to the specimen (fig. 21). The illumination so obtained is brighter than that given by the cover-glass illuminator, but is oblique, and, since part of the objective aperture is
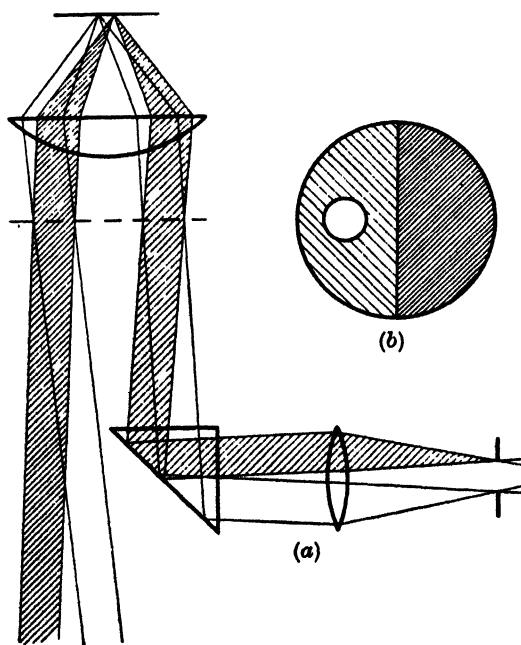
Fig. 21.—The prism illuminator.

(*a*) Schematic diagram of ray paths.
(*b*) Appearance of back of objective.

occluded, it is not possible to realize the maximum resolving power. There is also a rather disturbing sideways movement of the image when focusing. It is important with prism illuminators to use objectives in specially short mounts, as otherwise uneven illumination will be obtained due to the shadow of the reflector cast into the field of view.

Dark-Ground Illuminators for Opaques.

These are of two kinds, the catoptric, of which a well-known form is that due to Chapman and Alldridge (British Patent 215,979) (fig. 22), and the dioptric, such as is used in the Leitz Ultrapak (British Patent 373,080) (fig. 23). In each case, the illuminating beam is projected on to an annular reflector and thence
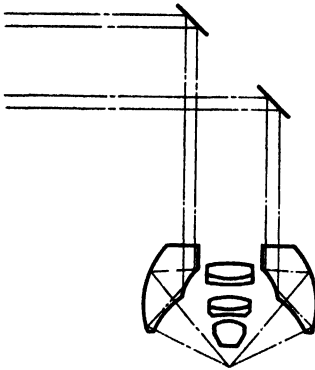
Fig. 22.—Chapman and Alldridge type.   Fig. 23.—Ultrapak type.
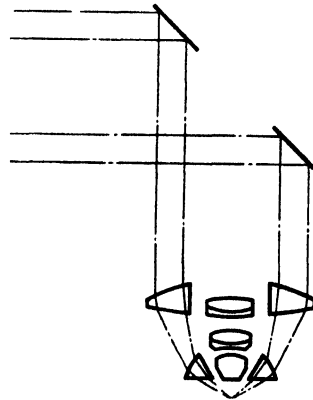
Dark-ground illuminators for opaques.

passes to the optical system of the illuminator which encloses the objective system. The incident light does not traverse the objective, hence the equivalent of dark-ground illumination is obtained.

Illuminators of this type are extremely useful but rather expensive, since several objective units are usually required to cover a complete range of powers. They are available for oil immersion as well as dry lenses.

Universal Illuminators.

It is convenient to be able to incorporate normal incident illumination from a glass reflector, " prism " illumination and dark-ground illumination in the one unit. One such device is shown in fig. 24a equipped for dark-ground illumination, and in fig. 24b for normal incident illumination with a glass reflector. By pushing the knob on the right the glass reflector is replaced by an inclined polished metal tongue which gives rise to prism illumination. In this case the source is mounted externally, but this is not essential and some types have been designed to incorporate the lamp unit. New systems of this kind have been described recently by Benford (1947).

(a)   (b)
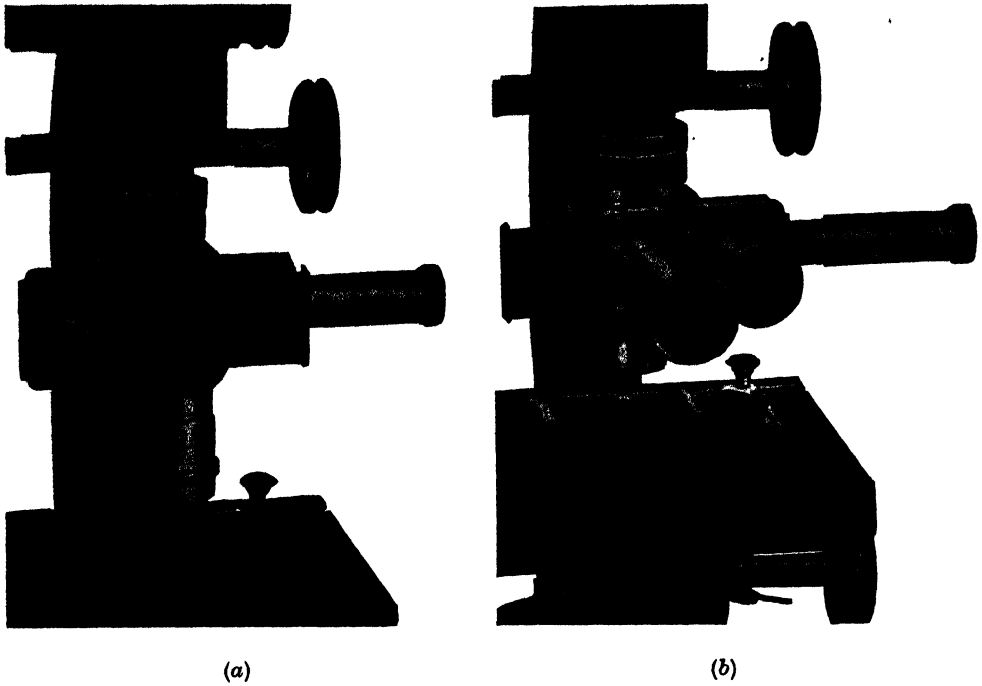
Fig. 24.—Universal illuminator for opaque specimens.

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DESCRIPTION OF PLATES.

PLATE I.—Photographs of paths of rays emerging from microscope condensers into
uranium glass

Fig. 1.—Two-lens Abbe, full aperture.
Fig. 2.—   „   „   reduced aperture.
Fig. 3.—Three-lens Abbe, full aperture.
Fig. 4.—   „   „   reduced aperture.
Fig. 5.—1·3 N.A. achromatic, full aperture.
Fig. 6.—1·0 N.A. universal, full aperture.
Fig. 7.—Bispherical dark ground, full aperture.

XIX.—THE EFFECT OF IODOACETAMIDE UPON CELL DIVISION 576.355 IN TISSUE CULTURES OF THE CHICK EMBRYO.

By A. F. W. HUGHES

(Sir Halley Stewart Research Fellow, Strangeways Research Laboratory.)

TWO PLATES AND ONE TEXT-FIGURE.

INTRODUCTION.

THE study of living cells is greatly aided by the use of the phase-contrast micro-scope (Taylor, 1946). For instance, it is possible directly to study in detail dividing cells in tissue tultures. When this technique is combined with serial photography, records can be obtained which give new information about the mitotic process (Hughes and Swann, 1948 ; Hughes and Preston, 1949). The technical details of these research methods have recently been described by Hughes (1949).

When the distortion of the normal mitotic process by a chemical (or physical) agent is being studied, only by these means can the sequence of events in an abnormal division be traced with certainty. The effect of mustard gas on mitosis in the chick has been investigated in this way by Hughes and Fell (1949).

The present paper describes the effects of iodoacetamide (IAM) on cell division in the same material. This work was undertaken at the suggestion of the late Dr. Louis Rapkine, who kindly supplied 0·5 gm. of the substance, which has been used throughout the experiments.

These observations are all of " short-term " effects on the cells : that is to say, the substance is added to a well-grown culture during or immediately before a particular cell enters division, and the subsequent effects on that cell are observed for a period of not more than 1 hour. Such a procedure is to be distinguished from that used in the study of the influence of mustard gas, cited above, where the agent is mixed with the culture medium at sub-cultivation and the observations are not made until 24 hours or so later, so that the effects seen may have been induced at any time during the whole period, probably long before the particular cell entered division.

The concentrations of IAM used in this work are lethal to cells in culture after 2 hours' application.

Material and Methods.

The tissue cultures were all original explants of frontal bone of 9–11 day chick embryos. They were explanted into a mixture of 1 drop of fowl plasma and 1 drop of embryo extract. I am greatly indebted to Mr. L. J. King,

technician at the Strangeways Research Laboratory, for preparing these cultures throughout the course of the work.

The culture chamber devised for these experiments is illustrated in text-fig. 1. It consists of a chromium-plated brass " ring," on either side of which 1-inch square cover-slips are cemented with paraffin wax. The upper of these bears the culture and medium as a " hanging-drop " preparation. Through the ring is drilled a fine hole 0·7 mm. in diameter, which at first is sealed with paraffin wax. A No. 17 hypodermic needle attached to a pipette charged with a solution of IAM in Tyrode's solution is introduced through this hole. The culture chamber and pipette are clamped to a brass plate, which is mounted on the stage of the microscope.
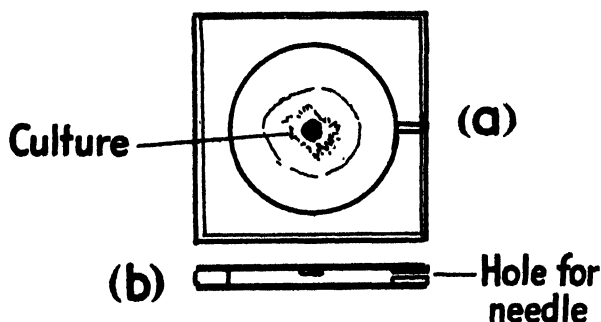
Fig. 1.

In this way, the culture can be grown normally and in contact with a free air surface, and the total thickness of the preparation is within the working distance in air of the phase condenser. With the culture and pipette in position on the stage of the phase microscope, a cell in the margin of the outgrowth at the desired stage of mitosis is selected, and the culture is then irrigated with IAM-Tyrode, and the observation is continued after a brief readjustment of the sub-stage condenser. It is not necessary to fill the whole volume of the culture chamber, and the saline can be kept away from the metal wall to avoid the possibility of contamination by metallic ions. If desired, the fluid can be withdrawn and the observation then resumed.

The volume of the saline introduced into the culture chamber is large in comparison with that of the culture and its medium. If equilibrium between fluid and medium were established without reaction between them, the final concentration in the latter would not be much less than that in the added reagent, but in fact reaction with both medium and tissue reduces this concentration to an unknown extent.

The fluid was either left in contact with the culture for the whole period of the experiment, up to 1 hour in duration, or was withdrawn after a few minutes before the observations began.

By means of this technique, a cell can be observed in mitosis and a reagent applied at any stage of the process. It is desirable to use such a method where an agent exerts more than one inhibitory action on mitosis. Thus, the telophase effect here described below cannot be observed unless anaphase has begun before

the IAM is present in effective concentration so that the cell has escaped the inhibition of metaphase.

The phase contrast objectives and condenser were supplied by Messrs. Cooke, Troughton and Sims, Ltd., of York. Full details of their use in ciné-micrography will be found in the paper by Hughes (1949) already cited.

Fixing and Staining.

The methods were described in Hughes and Fell (1949). At the end of an experiment, the position of the observed cell in the outgrowth of the culture was sketched and the culture was fixed in Maximov's solution (Zenker without acetic was used in the earlier part of the work). Staining was either by Feulgen's technique or with Ehrlich's hæmatoxylin after hydrolysis. The cultures were mounted whole in the usual way. The particular cell was then identified in the permanent preparation and compared with its photographs in life.

I am very grateful to Dr. H. B. Fell for staining and mounting all the material used in this work.

<center>OBSERVATIONS.</center>

Normal Mitosis in the Chick.

The general features of normal mitosis in the chick as seen in the phase microscope have been described by Hughes and Fell (1949). Recently these observations have been extended to early prophase, for after much practice it is possible to identify cells at this stage and follow them through the rest of the mitotic cycle.

In a cell in early prophase the " background " of the nucleus is faintly granular, due to the gradually increasing charge of nucleic acids on the interphase chromonemata. As the chromosomes become more distinct, many of them are seen to be attached to the nucleoli by the heterochromatic chromocentres with which the chromosomes are continuous. During prophase, slight movements are seen within the nucleus.

Towards the end of prophase the nucleoli and the nuclear membrane suddenly vanish, at about the same time. The chromosomes then take up the radial arrangement described in Hughes and Fell (1949). The remainder of the mitotic process is described in this paper.

The maximum duration of metaphase is greater than that given in the previous paper. Metaphase tends to be prolonged in those cells in which most of prophase has been followed under the microscope. The change from prophase to metaphase is always a very indefinite one.

Saline Controls.

Before the experiments with IAM were begun, the effect of flooding with Tyrode on the course of mitosis was studied. Preliminary observation showed that cells would divide normally for 1 hour and often for 2 hours after the culture had been covered with Tyrode. After periods longer than 1 hour, however, slight irregularities in division began to be observed, such as increased bubbling in anaphase and a distorted cleavage. In cultures fixed after flooding

15

for 1 hour or more, rather distorted prophases can be seen. Since it was not found necessary to prolong the experiments with IAM for more than 1 hour, it was considered that flooding of the culture *per se* could have very little effect on the experimental results.

In later experiments, the cultures were covered with Tyrode for 5 minutes only. Control cultures treated in this way were indistinguishable from untreated controls when examined several hours later. In fixed preparations of normal tissue cultures there are always a few slightly abnormal mitotic figures to be seen. In each batch of experiments at least one saline control was included, which had been flooded for the same time as in the experimental series. In this control culture, one cell was usually followed through mitosis.

The Effects of Iodoacetamide.

(1) *At Metaphase and Anaphase.*—In the first series of experiments, the effect on cells in metaphase of IAM at concentrations of $10^{-3 \cdot 3}$ M. in Tyrode was examined. In some, mitosis did not proceed any further. The spindle was disorganized, the metaphase plate became disarranged, and the chromosomes clumped together. In others, where the IAM had been applied towards the end of metaphase, anaphase was not inhibited and proceeded normally. In some of this group cleavage of the cell took place, but in others was inhibited.

When the IAM is applied to the culture, the substance diffuses through the plasma gel, reaches the cell, and gradually increases in concentration inside. If the inhibitory concentration is not reached before the end of metaphase, anaphase begins and in all but one recorded instance, proceeds normally. This fact suggests that at any rate the latter part of anaphase can take place in the presence of a concentration of IAM sufficient to disorganize the metaphase spindle.

Since these results are generally so clear-cut, they can be used to estimate the time necessary for an effective concentration of IAM to build up inside the cell. In a series of cells treated in metaphase, the maximum time observed between the application of the agent and the onset of anaphase will be approximately equal to this time. The results given in Table I suggest that at an applied concentration of $10^{-3 \cdot 3}$ M., this is 2–3 minutes, and at $10^{-3 \cdot 6}$ M. of the order of 7 minutes. The relative length of these two periods at these two concentrations is consistent with the above argument.

Normally in metaphase the chromosomes are in a state of random linear movement along the spindle (Hughes and Swann, 1948). After IAM has been applied to a cell early in metaphase, this movement ceases and the chromosomes then clump into a rigid mass, often roughly resembling a ring. The movement of the chromosomes into this formation can resemble the first few seconds of a normal anaphase. The point at which the chromosomes clump can be determined in the film records to within a minute or so, and the time between the application of IAM and the appearance of this reaction is given in Table I; this period is rather longer than the corresponding one in later metaphase for the suppression of the anaphase movement. Anaphase never occurs once the random movement of the chromosomes has ceased.

While the chromosomes are becoming clumped, the surface of the cell is
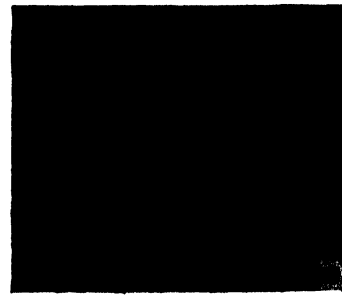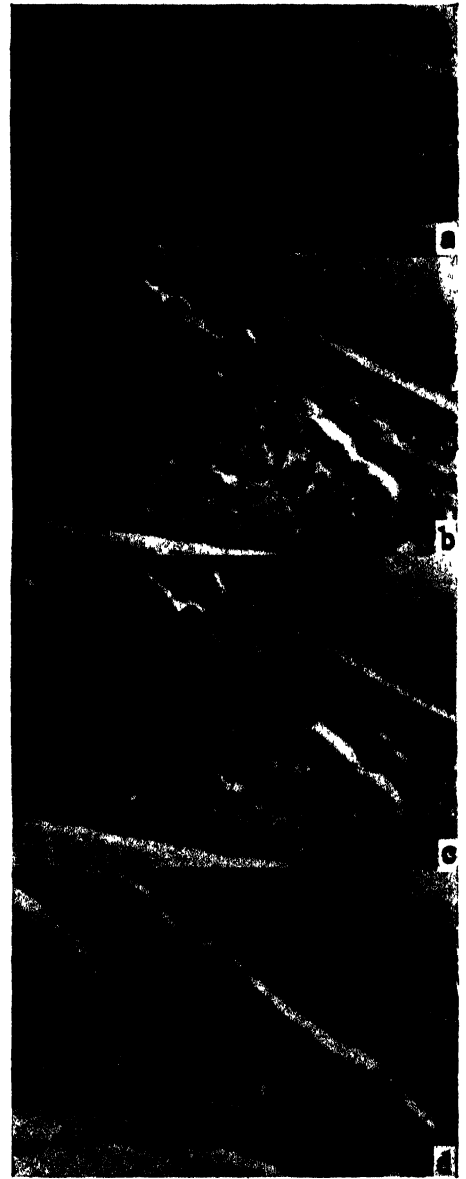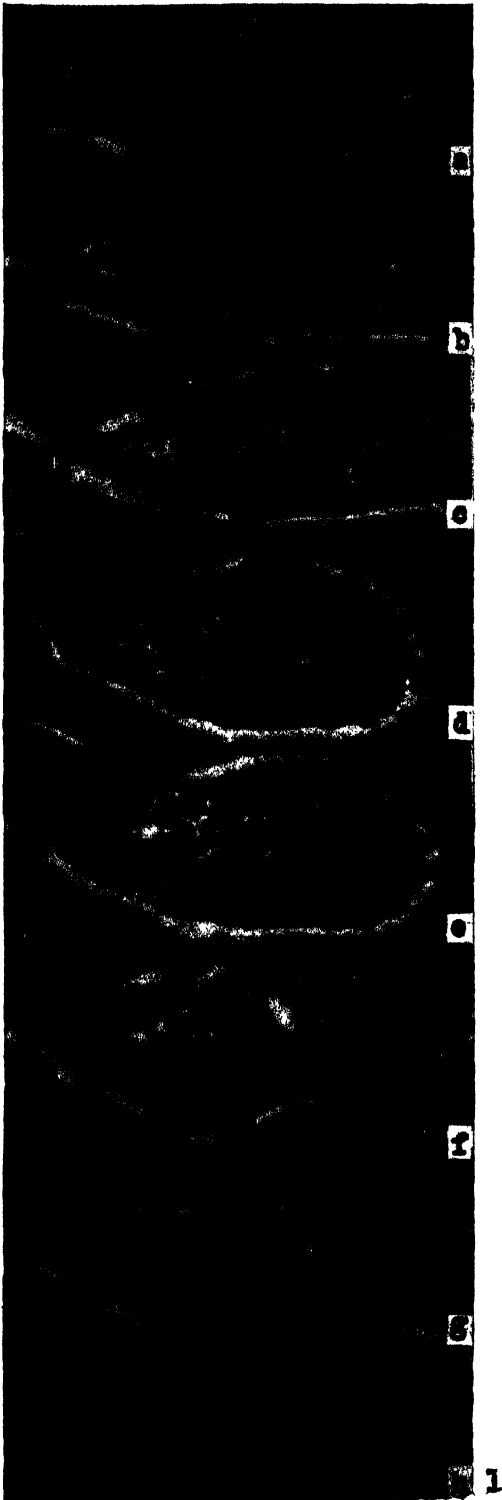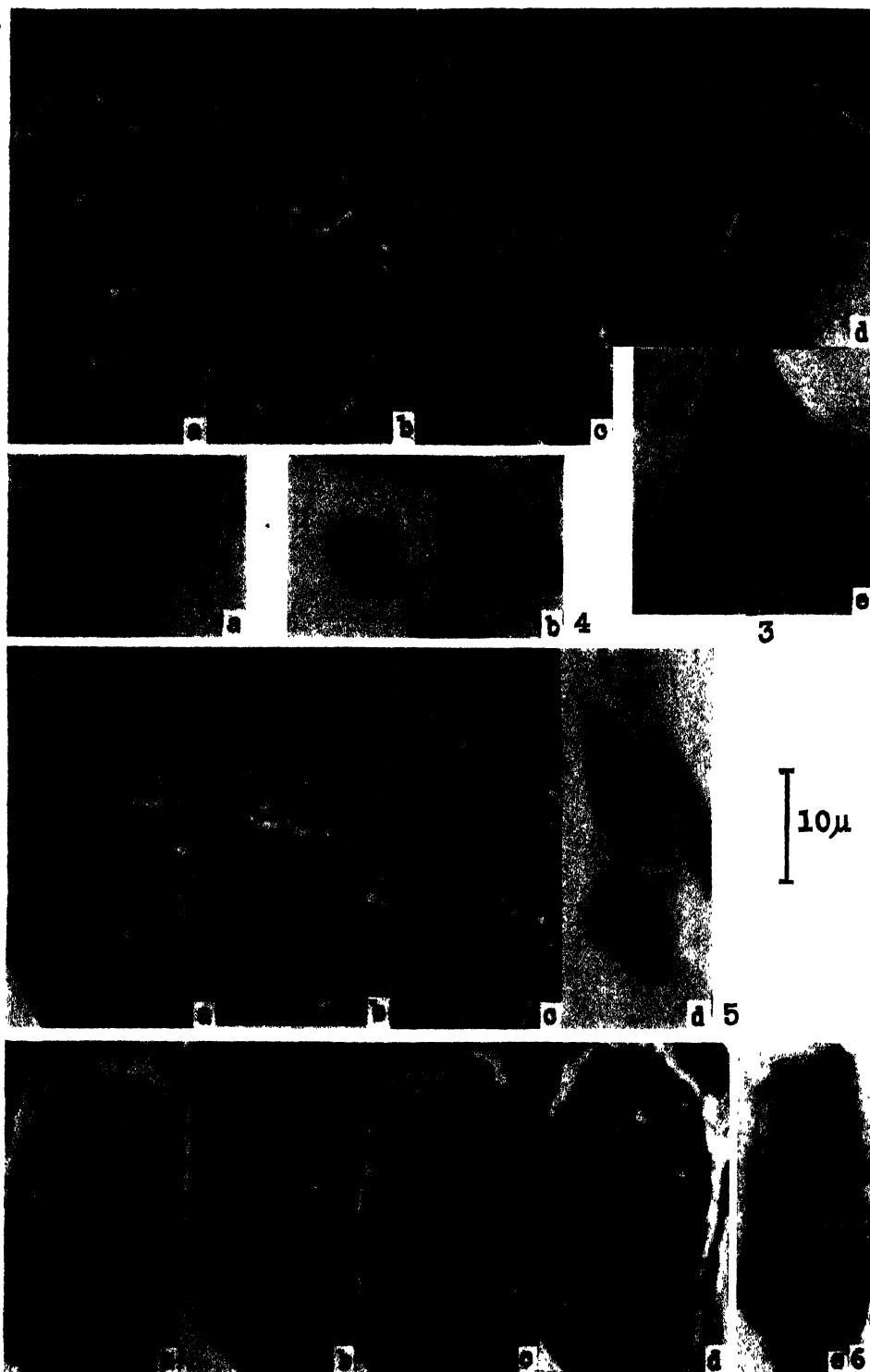
TABLE I.

Stage of mitosis.	Untreated controls.	Controls treated with Tyrode.	Treatment of iodoacetamide : concentration and time of application.	Result at given time after treatment.
Prophase. Time in record before nucleoli go.	(1) 19 min.	(1) 25 min.	10^{-4} M., 5 min.	(2) 23·4, 39 min.
Metaphase. Spindle inhibition.			$10^{-3\cdot3}$ M., T.R.	(6) R a n d o m linear movement ceases 3·7–8·5 min.
			10^{-4} M., T.R.	(1) Do. ceases 14·6 min.
Normal metaphase followed by anaphase but not cleavage.			$10^{-3\cdot3}$ M., T.R.	*(1) Anaphase begins 3 min.
			$10^{-3\cdot6}$ M., T.R.	*(10) Anaphase begins 1–7 min.
Normal metaphase followed by anaphase and cleavage.	Metaphase lasts (11) 4–17 min.	Saline on T.R. (6) 3–20 min.	$10^{-3\cdot3}$ M., T.R.	*(2) Anaphase begins 1–3 min.
			$10^{-3\cdot6}$ M., T.R.	*(4) Anaphase begins 3–7 min. *(1) Imperfect anaphase 7½ min.

No. of examples indicated by brackets.
T.R. = (throughout record).
* Show subsequent telophase inhibition.

often thrown into violent turmoil, as seen in a speeded-up film. Bubbling at the cell surface graduates into a kind of swaying motion of the cell from side to side. Gradually this subsides into a " waggle " of the cell at one end or the other. Finally the cell becomes still and the inclusions move very little. Thus at the end of this stage, ½–1 hour after the application of the IAM, the protoplasm still appears normal and entirely uncoagulated. With high concentrations of IAM ($10^{-3\cdot3}$ M.), in adjacent intermitotic cells, the lipid ˙ granules are seen in abnormally active Brownian movement after this period.

If both anaphase and cleavage occur, the turmoil during the latter process seems fantastic in its violence in the speeded-up films. The cleavage is usually greatly distorted and often incomplete. The daughter cells do not flatten out into the normal interphase form.

(2) *On Nuclear Reconstruction.*—When anaphase occurs under the influence of IAM, it is not followed by a normal telophase. The inhibition of this process takes various forms, in all of which the chromosomes are wholly or partly prevented from assuming their interphase condition. The chromosomes can (a) remain clumped in a tight knot, or (b) they may greatly expand and loosen, without the formation of the nuclear membrane and nucleoli. In less severely inhibited daughter nuclei (c), a nuclear membrane develops which may enclose both nucleoli and Feulgen-positive chromosomes (pl. II, fig. 4).

In condition (b), the chromosomes form irregular elongated loops, which in one cell were clearly seen to consist of double threads (pl. II, fig. 6d).

Daughter nuclei of type (c) are often larger than normal early interphase nuclei, and might be taken for early prophase but for the fact that they occur in pairs (pl. II, fig. 4).

When concentrations of IAM of the order of 10^{-4} M. are applied to chick cultures, the inhibition of telophase seen 40–60 minutes later, may be of types (a), (b), or (c). With stronger concentrations type (a) becomes more frequent. A more important factor in determining the type of effect, however, is the point in the mitotic cycle at which the reagent is introduced. If approximately 10^{-4} M. IAM is added to a culture when a given cell is in early anaphase, an inhibition of type (a) will generally result (pl. II, fig. 6) ; if treatment is delayed until the cell is in cleavage, the chromosomes will be partly retained, and nucleoli will be formed (pl. II, fig. 5).

The interval between the addition of the reagent to a culture and the arrival inside a cell of a concentration sufficient to interfere severely with nuclear reconstruction must be of the order of several minutes ; at an applied concentration of 10^{-4} M. ; this period is possibly greater than the normal interval between anaphase and the end of cleavage, namely 4–6 minutes (Hughes and Fell, 1949). Usually, where an effect of type (a) or (b) is produced, cleavage of the cell is also inhibited, as in the example illustrated in pl. II, fig. 6.

(8) *On Prophase.*—Since nuclear reconstruction is, broadly, the reverse of the events in prophase, it is of interest to enquire whether the dissolution of nucleoli and the formation of the chromosomes can also be inhibited by the same concentrations of IAM.

IAM at concentrations of 10^{-4} M. was applied for 5 minutes to standard cultures, which were then searched for early prophases. On two occasions, a prophase was found well before the nucleoli had gone (data in Table I). During subsequent observation both the formation of the chromosomes and the dissolution of the nucleoli and nuclear membrane took place normally. The only abnormality was the failure to form a mitotic spindle, and the consequent lack of the normal movements of the chromosomes at the end of prophase. After the nuclear membrane had gone, the chromosomes thickened and clumped into an immobile mass.

On each occasion the time at which the nucleoli disappeared was between 25 and 30 minutes after the first application of the IAM, a period much longer than that estimated as that necessary for a concentration to build up inside the cell sufficient to inhibit the spindle at metaphase. It appears, therefore, that at concentrations of this order IAM does not affect prophase before the disappearance of the nuclear membrane. This conclusion is supported by the study of stained preparations of cultures fixed 40–60 minutes after treatment with IAM at concentrations which cause metaphase and telophase inhibitions. In such preparations, cells in the stage of prophase just after the disappearance of the nucleoli are often seen, with the chromosomes normal in arrangement and appearance. Such cells have presumably entered prophase in the presence of the reagent, but the clumping action of the IAM on the chromosomes has not yet occurred before fixation.

The Minimum Effective Concentration of IAM.

In two separate experiments batches of cultures were treated with successive dilutions of IAM and then fixed at periods between 45 and 60 minutes afterwards. In both the border-line concentration was found to be 10^{-5} M., approximately.

In four cultures treated for 5 minutes with IAM at this concentration and fixed 50 minutes later, normal stages of mitosis were observed, including some early anaphases. In two of these cultures there were signs of metaphase and telophase inhibition.

Three cultures were treated with $10^{-4.6}$ M. IAM, under the same conditions, and fixed at the same time after treatment. Only in one were normal mitotic figures seen, and all three showed both types of inhibition.

The Effects of IAM on Intermitotic Cells.

As mentioned above (p. 215) cultures treated with IAM, at all concentrations used, are wholly abnormal 2 hours after application.

At the end of the first hour, the only abnormalities then visible are the abnormally active Brownian motion of the fat granules in the cytoplasm, and the appearance of blebs at the surface of some cells. During the next hour, these blebs increased in number and extent. Finally, after 2 hours, the cell consists of a mass of vesicles, attached to a shrunken centre, in which is a largely pycnotic nucleus.

None of the experiments described in this paper extended more than one hour beyond the addition of the reagent. Only at the higher concentrations of IAM were blebs just appearing at the surface of the inter-phase cells during the period of the experiments.

Comparison of the Effects of Colchicine with those of IAM.

The action of iodoacetamide on the inhibition of the spindle resembles the well-known effect of colchicine on animal cells. Experiments were made to find whether colchicine also exerts an inhibitory effect on telophase.

Ludford (1936) found that colchicine could arrest cells in metaphase in tissue cultures of both mouse carcinomata and embryonic kidney when applied at the immense dilution of 1 : 100,000,000 ($10^{-8.4}$ M.).

Colchicine dissolved in Tyrode's saline was applied for 5–6 minutes to chick osteoblast cultures at the much lower dilution of $10^{-6.4}$ M. A cell was then selected in which anaphase had just begun as the colchicine reached it. The camera was started and the record continued for 30 minutes, by which time, the daughter cells were fully in interphase. Three such observations were made in all of which telophase and reconstruction proceeded entirely normally. Both in phase-contrast immediately before fixation and after staining with hæmatoxylin it could be seen that in the daughter nuclei, nucleoli had developed, and that the interphase chromonemata and chromocentres had been formed from the chromosomes (pl. II, fig. 3).

In metaphase there is also a difference in the appearance of cells inhibited by these two reagents. With colchicine the chromosomes themselves look

perfectly normal, and their split condition can be readily recognized. There is none of the clumping action of IAM.

Forty minutes after the $10^{-6.4}$ M. colchicine had been applied to a culture for 5 minutes, the lipoid granules in interphase cells were in active Brownian movement. After 4–5 hours most of the cells had lost their normal appearance and had become rounded.

DISCUSSION.

Iodoacetamide reacts both with sulphydryl groups (Rapkine, 1933, *a* and *b*) and amino-groupings (Barron and Singer, 1945). Among the biological effects of sulphydryl reagents, their action on dividing cells has been frequently studied and the literature on this subject is well reviewed by Brachet in his " Embryologie chimique " (1947, p. 184). Interest in this field has largely centred round the views of the late Dr. Louis Rapkine on the reversible denaturation of proteins during the mitotic cycle and the influence of sulphydryl reactants on this process. Sulphydryl groups, Rapkine suggested, act on proteins through intermediate reactions with reduced and oxidized glutathione.

The evidence for this theory comes from studies on the effects of sulphydryl reactants on mitosis. Failure to prevent cell division by treatment with monoiodoacetic acid, the agent mainly used in such studies, has been used as evidence against Rapkine's theory (Ellis, 1933). Monoiodoacetic acid reacts less rapidly with —SH groups than does iodoacetamide (Smythe, 1936) and the iodoacetate ion penetrates a living cell much more slowly than the non-ionic iodoacetamide.

The prevention of cell division by —SH reactants may well be due to spindle inhibition, such as is described in this paper. The theory of Rapkine could easily be applied to the mitotic spindle, but for the fact that its inhibition may be caused in so many ways other than by treatment with a sulphydryl reactant. Colchicine is remarkable for its effect at so great a dilution, but Östergren (1944) has shown that a similar result is produced by a wide range of substances, of different chemical composition. In chick cells in tissue culture, the spindle can be inhibited in many ways, for instance by strong light and temperature shocks. It seems unlikely that the physiology of the mitotic spindle could be assigned to any single physico-chemical action, although reversible denaturation of proteins may well be the basis of its formation and activity in anaphase.

It remains to be shown how far the inhibitory effect of IAM on telophase is specific for sulphydryl reactants. Although it is not given by colchicine at the concentration used in this work, other chemical agents may yet be found to interfere with nuclear reconstruction.

The present experiments have shown that the formation of the nucleoli in telophase is inhibited by a concentration of IAM which permits their dissolution in prophase. There are two possible reasons for this difference :

(1) The nuclear membrane is present during prophase until the nucleoli have largely disappeared. The intact nuclear membrane might have a low permeability to iodoacetamide, although this does not seem a likely explanation of the observation.

(2) Before the visible events of telophase can proceed, it may be necessary for chemical changes to take place which are sensitive to sulphydryl reactants, and which do not occur in prophase.

Further work is in progress in an attempt to shed further light on these questions.

SUMMARY.

1. A technique is described for the direct study of a chemical agent upon dividing cells in tissue cultures. The method is combined with phase-contrast cinémicrography.

2. The action of the sulphydryl reactant iodoacetamide has been studied by this means.

3. There are two effects on chick cells in mitosis: an inhibition of the mitotic spindle and of the reconstruction of the nuclei in telophase.

4. The metaphase effect is similar to but not identical with that of colchicine. This substance has no effect on telophase at the concentrations used.

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DESCRIPTION OF PLATES.

PLATE I.

Fig. 1 (a–g).—Normal mitosis of chick osteoblast in tissue culture. Phase-contrast. Enlargements from 16-mm. film record ×1600.

(a) Prophase, with nucleoli and nuclear membrane still present.
(b) 4 mins. after (a): nucleoli have just disappeared.
(c) 9 mins. after (a): "radial" stage.

(d) 31 mins. after (a) : near end of metaphase.
(e) 34 mins. after (a) : early anaphase.
(f) 39 mins. after (a) : cleavage in progress.
(g) 43 mins. after (a) : daughter nucleoli in reconstruction.　Nucleoli have just appeared.
(h) 56 mins. after (a) : in the daughter nuclei, nucleoli, and chromocentres are visible.

Fig. 2 (a–e).—The effect of IAM on prophase.　Approximately 10^{-4} M. IAM added to chick osteoblast culture for 5 minutes.　Film record begins 13 minutes later.　Photographic details as in Fig. 1.

(a) Nucleoli still present.
(b) 22½ mins. later than (a) : nucleoli about to vanish.
(c) 24 mins. later than (a) : nucleoli have gone.
(d) 49 mins. later than (a) : no spindle has developed.
(e) Culture fixed 65 mins. after film record began.　Stained in hæmatoxylin.　The chromosomes are still arranged as in (d).

PLATE II.

Fig. 3 (a–e).—The effect of colchicine on a chick osteoblast in anaphase.　Approximately $10^{-6.4}$ M. colchicine added to culture for 5 minutes.　Film record on a cell in anaphase begins 7 minutes later (a).　Photographic details as in Fig. 1.

(b) 5·5 mins. later than (a) : cleavage complete.
(c) 10 mins. later than (a) : nucleoli are appearing.
(d) 30 mins. later than (a) : lower daughter cell of (a–c).　Nucleoli and chromocentres in nucleus.
(e) Culture fixed 35 mins. after film record began.　Stained in hæmatoxylin.　Same daughter cell, as in (d).

Fig. 4 (a and b).—Telophase pairs, showing chromosome retention in a chick osteoblast culture, treated with approximately $10^{-3.6}$ M. IAM which remained in contact with the culture until fixation, 45 minutes later.　Stained by Feulgen's method.　×1600.

Fig. 5 (a–d).—The action of IAM on a chick osteoblast in anaphase.　Weaker effect.　Photographic details as in Fig. 1.　Approximately 10^{-4} M. IAM added to culture for 5 minutes.　Film record on cell in anaphase begins 3 minutes later (a).

(b) 5·5 mins. later than (a).
(c) 18 mins. later than (a) : daughter nuclei show some chromosome retention.
(d) Culture fixed 55 minutes after film record began.　Stained in hæmatoxylin.　Nucleoli and chromosomes in daughter nuclei.

Fig. 6 (a–e).—The action of IAM on a chick osteoblast in anaphase.　Stronger effect.　Photographic details as in Fig. 1.　Approximately $10^{-3.6}$ M. IAM added to culture for 8 minutes.　Film record on cell in anaphase begins 11 minutes later (a).

(b) 4 mins. later than (a).
(c) 7 mins. later than (a) : cleavage has been suppressed.
(d) 25 mins. later than (a).　Chromosomes still present as double threads.
(e) Culture fixed 47 mins. after film record began.　Stained in hæmatoxylin.　Full chromosome retention.

XX.—NOTES ON SOME DIATOMS FROM NORFOLK. 582.61

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In the course of œcological work on the microscopic algæ of the salt-marshes at Blakeney Point, Norfolk, which one of us (Abdin) has been carrying out, three new species of diatom have been found, and also another for which there is no validly published name. These are described below, and there is a discussion of the name to be applied to a further species for which that in current use is incorrect. A general account of the area from which the gatherings were made is given by Oliver and Salisbury (1913).

Nitzschia irregularis spec. nov.

Valvis lanceolatis, capitatis, 24μ–35μ longis, 4·5μ–5μ latis ; carina excentrica, canaliculis (punctis carinalibus) latis, irregulariter dispositis, non prolongatis, duobus centralibus non distantioribus quam aliœ, 6–7 in 10μ ; striis 32–33 in 10μ ; frustulis subrectangulis, sed polos versus leviter fastigantibus, angulis rotundatis ; cingulis simplicibus, plicaturis longitudinalibus 2 vel 3. Habitat in salinas ad Blakeney Point, Norfolk.

Pl. I, fig. 1.

Typus in Herb. Mus. Brit., coll. Diat. no. B.M. 36133.

Valves lanceolate, capitate, 24μ–35μ long, 4·5μ–5μ broad ; keel excentric, its canaliculi (carinal puncta) broad, irregularly spaced, not prolonged, the two central ones not more distant than the others, 6–7 in 10μ ; striæ 32–33 in 10μ ; frustules sub-rectangular but tapering gently towards the ends, corners rounded ; girdle simple with two or three longitudinal folds.

This species is a member of the section *Lanceolatœ* Grun. apud Cleve and Grun. It is distinguished from the other members of that section by possessing distant, broad, and irregularly spaced carinal canaliculi together with fine striæ and capitate apices. The spacing of the carinal canaliculi has suggested the specific epithet. *N. frustulum* Kütz. has a very similar keel, but its ends are not capitate and its striæ are much coarser. The form which Hustedt (1939) has described and figured as *N. frustulum* Kütz. var. *perminuta* Grun. apud Van

Huerck forma *constricta* Hust. has the same density of striæ and possesses sub-capitate apices, and it would appear from the figure that its carinal canaliculi are also irregularly spaced. They are, however, much closer than those of *N. irregularis* Ross and Abdin, and Hustedt's form also has the valves slightly constricted in the centre. It is probable that his form is closely related to our species, and that Hustedt (1939) is justified in doubting whether he is correct in attributing it to *N. frustulum* Kütz.

N. irregularis Ross and Abdin is found all the year round in pans in the *Pelvetia-Salicornia* marsh. These pans are of two types, those with a hard floor and those with a floor of soft mud, and the species occurs only in those with a hard floor. The salinity of these pans is usually close to that of sea-water and it can therefore be classed as euhalobous and, like all salt-marsh forms, euryhaline.

Dr. Hustedt, of Plön, Germany, to whom material containing this species was sent, wrote that it was unknown to him and apparently new. We are grateful to him for his opinion, which confirms our own.

Surirella minima spec. nov.

Valvis ovato-cuneatis, apicem inferiorem obtusum versus attenuatis, 18μ–20μ longis, 8μ–10μ latis ; alis validis, canaliculis c. 6 in 10μ, tam latis quam spatia interjacentia sunt ; pseudocostis distinctis ; pseudorhaphe lineari ; sine striis visibilibus. Habitat in salinas ad Blakeney Point, Norfolk.

Pl. I, fig. 2.

Typus in Herb. Mus. Brit., coll. Diat. no. B.M. 36136.

Valves ovate-cuneate, heteropolar, obtusely pointed at the lower apex, 18μ–20μ long, 8μ–10μ broad ; alæ well developed, alar canals about 6 in 10μ, about as broad as the intervening spaces ; pseudocostæ distinct ; pseudoraphe linear ; no visible striæ.

This species resembles *S. tenera* Greg. in form and structure, but differs from it so markedly in size and in the spacing of its pseudocostæ, as well as in habitat, that it must be regarded as completely separate. Other closely related species are *S. tenuis* Mayer, which, however, is much more linear, as well as larger and with more distant pseudocostæ, and *S. Oestrupii* Gran, which also has the pseudocostæ more distant.

S. minima Ross and Abdin occurs sporadically, and never in any considerable numbers, in collections from the main creek in the salt marsh at Blakeney Point. It has been found at varying places along the creek, but mostly from mid-tide levels, particularly where the bottom is sandy, although it is present also on shingle and mud. From its wide range of level and the position of its greatest abundance it is to be classed as mesohalobous and euryhaline. It appears to be favoured by warm conditions and has been found only between May and October.

Surirella hispida spec. nov.

Valvis ovatis, apicem unum versus attenuatis, 35μ–70μ longis, 23μ–37μ latis ; alis invalidis, canaliculis 1½–2½ in 10μ ; superficie valvæ distincte undulata, undis
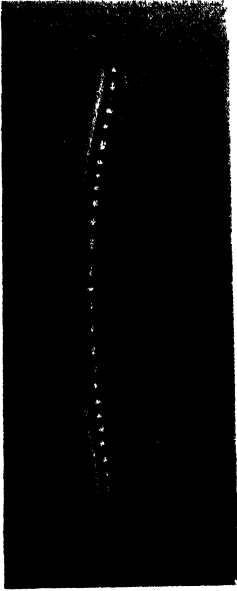
Fig 1
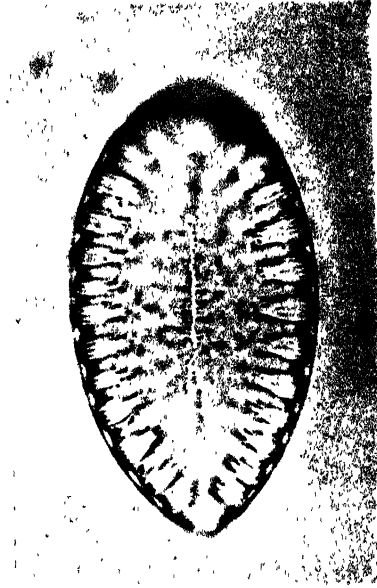
Fig 3

Fig 2

Fig 4

pœne radiatis, lacunis suis prope marginem et pseudorhaphen linearem angustis-simis, tam latis quam summœ undœ in depressione longitudinali inter marginem valvœ et pseudorhaphen, quarum utraque in planitie eadem est ; striis c. 14 in 10μ, in summis undis contentis nisi apud marginem et pseudorhaphen, distinctis in parte exteriori valvœ, indistinctis in depressione longitudinali, ubi summœ undœ spinis sparsis statœ sunt ; frustulis a latere cinguli visis cuneatis. Habitat in *salinas ad* Blakeney Point, Norfolk.

Pl. I, fig. 3.

Typus in Herb. Mus. Brit., coll. Diat. no. B.M.36135.

Valves ovate, heteropolar, 35μ–70μ long, 23μ–37μ broad ; alæ poorly developed, alar canals 1½–2½ in 10μ ; surface of the valve distinctly undulate, the undulations nearly radiate, their hollows very narrow close to the margin and the linear pseudoraphe, as broad as the crests in the longitudinal depression between the margin and the pseudoraphe, which are both at the same level ; striæ about 14 in 10μ, confined to the crests of the undulations except very near to the pseudorhaphe and margin, distinct in the outer part of the valve, indistinct in the longitudinal depression, where the crests of the undulations bear scattered spines ; frustules in girdle view cuneate.

? *Surirella ovulum* Hustedt in Int. Rev. Hydrobiol. **42**, 164, f. 411 (1942) *nomen nudum sine descriptione latina.*

The striæ in this species have the appearance of being spinulose, since the silica of the valve is rather thick and the puncta run obliquely through it. This gives it the appearance to which its specific epithet refers, and, along with its smaller size, at once distinguishes it from *S. striatula* Turp., in which also the striæ are confined to the crests of the transverse undulations of the valve. It also very closely resembles *S. caspia* Brun, which is larger and has the pseudo-raphe and valve somewhat curved.

Dr. Hustedt, who has seen specimens of this species, writes that it is identical with his *S. ovulum* Hust. However, the single specimen of that diatom from a fresh-water lake on the Philippine Island of Cagayan-Sulu appears from his figure and description to have a well-defined raised lanceolate area in the centre, whilst in our species the pseudoraphe is raised but not sur-rounded by such an area. Hustedt's species also has the alar canals closer, 3–4 in 10μ, against the 1½–2½ in 10μ which is the full range found in the many speci-mens of our species which we have examined. These differences, together with the difference in habitat, makes us not completely certain that the two species are identical. For this reason we have preferred to use a new name for our species rather than to adopt and validate Hustedt's, which, being accompanied by no Latin diagnosis, is technically a *nomen nudum.*

S. hispida Ross and Abdin is frequent in gatherings from a creek with a shingle bottom about the middle of the marsh. The length of time for which the area is covered during each tide averages about 4 hours. There is a seepage of fresh water through the shingle and hence conditions vary from brackish to fully salt. The diatom is also not infrequent in another creek rather higher on the marsh, whose bed is of coarse brown sand. It therefore seems that it is to be classed œcologically as mesohalobous and euryhaline.

Campylodiscus fastuosus Ehrenberg in Ber. preuss. Akad. Wiss., **1845**, 361 (1845).

Campylodiscus parvulus W. Smith in Ann. Mag. nat. Hist., ser. 2, **7**, 7, pl. 1, f. 4 (1851) *non auct.*

Campylodiscus Thuretii Brébisson in Mém. Soc. Sci. nat. Cherbourg, **2**, 251, f. 3 (1854).

Campylodiscus simulans Gregory in Trans. microsc. Soc. London, n.s., **5**, 77, pl. 1, f. 41 (1857).

Campylodiscus bicruciatus Gregory, l.c., 78, pl. 1, f. 42 (1857).

This is a common and variable species with a wide range in size, but in the gatherings from Blakeney Point only the smaller forms have been met with. Its distinguishing feature is the presence of two rounded ridges running from one end of the valve to the other; these ridges and the linear or narrowly lanceolate space between them are covered with transverse striæ about 12–13 in 10μ. In the space between there are often a few strong and irregularly placed transverse lines. Immediately outside the ridges the pseudocostæ are narrow and definite, appearing as short lines; at a small distance from the ridges, however, they expand into narrowly campanulate striate elevations. The full range of structure found in the species is shown by H. and M. Peragallo (1897–1908, pl. 57, f. 4–9). It is a common species of world-wide distribution.

The species was first described by Ehrenberg on material from the East Indies, but he gave no figure and his diagnosis was brief. It did, however, include the comment " *Surirellæ fastuosæ affinis* " which is applicable to no other species of *Campylodiscus* Ehrenb. Examination of W. Smith's type specimen shows that his *C. parvulus* was certainly this species, as can be clearly seen from his later figure (W. Smith, 1853, pl. 6, f. 56). The original one was very sketchy and almost unrecognizable. Smith's species was misinterpreted by Grunow (1862, p. 445, and in Van Huerck, 1880–5, pl. 77, f. 2), who used the name for *C. innominatus* Ross and Abdin (q.v. below). This error was repeated by Van Heurck (1896) and H. and M. Peragallo (1897–1908), and this may be regarded as the current sense in which the name is used. De Brébisson, who found specimens of a larger form of the species near Cherbourg, described them as new under the name *C. Thuretii* Bréb. and accompanied his description by a very poor and inaccurate figure. It is only possible to ascribe his name to this species on the basis of Gregory's (*l.c.*) statement that his *C. simulans* " is the same as his (De Brébisson's) *A.* (sic !) *Thuretii*. But as I had found it and named it, and even communicated it to M. de Brébisson long before his paper appeared I retain my own specific name." *C. bicruciatus* Greg. is shown by specimens in coll. R. K. Greville, who drew Gregory's figures, to have been founded on a complete frustule of the species, as Cleve (1883, p. 482) first recognized, although Gregory suspected that he might be dealing with a frustule and not a single valve.

Almost all authors have agreed that *C. fastuosus* Ehrenb., *C. Thuretii* Bréb., *C. simulans* Greg., and *C. bicruciatus* Greg. are the same species. Apart from Ralfs (in Pritchard 1861) and Grunow (1862) they have not used Ehrenberg's name, in spite of its priority, presumably because they felt some uncertainty

as to its application owing to the fact that he gives no figure. As is pointed out above, his description seems to us sufficient to enable his name to be used for the species with some certainty. *C. parvulus* W. Smith, however, has generally been regarded as a separate species, either because the specific identity of the large and small forms of the species was not recognized, or because of the error in the ascription of the name already referred to. Deby (1891), however, in his treatment of the genus, recognized that *C. parvulus* W. Smith was to be ascribed either to *C. Thuretii* Bréb. or to *C. simulans* Greg., which two species he separated with some doubt and, as Gregory's remark quoted above shows, no justification. This discussion will make clear our reasons for considering that this species should be known as *C. fastuosus* Ehrenb. and that the other names mentioned should be relegated to its synonymy.

At Blakeney Point the diatom has been collected from a number of stations in the main creek and its tributaries. It is also recorded from many marine localities and is therefore to be classed as euhalobous and euryhaline in its ecological requirements.

Campylodiscus innominatus spec. nov.

Valvis parvis, 25μ–50μ diametro, fortiter flexuosis ; pseudorhaphe angusta, lineari, hyalina ; lineis sublatis duabus distinctis ab loco prope apicem utrumque rapide divergentibus, deinde cum pseudorhaphe pœne parallelis et circa partem tertiam radii valvœ ab ea distantibus ; costis linearibus, ad marginem 4, ad pseudorhaphen 8 in 10μ, continuis a pseudorhaphe ad marginem, ubi ad faciendos canaliculos parvos ovales, inter quos striœ breves nullœ sunt, extendunt ; sine striis visibilibus in superficie valvœ. Habitat in salinas ad Blakeney Point, Norfolk.
Pl. I, fig. 4.

Typus in Herb. Mus. Brit., coll. Diat. no. B.M. 36140.

Valves small, 25μ–50μ in diameter, strongly flexed ; pseudoraphe narrow, linear, hyaline ; two narrow, well defined ridges diverging rapidly from a point near either apex, then becoming almost parallel to the pseudoraphe at about one-third of the radius of the valve distant from it ; costæ 4 in 10μ at the margin and 8 in 10μ at the pseudoraphe, continuous from the pseudoraphe to the margin where they expand to form small oval alar canals, between which there are no short striæ ; no visible striæ on the surface of the valve.

" *Campylodiscus parvulus* W. Smith " Grunow in Verh. zool.-bot. Ges. Wien, **12**, 445 (1862) ; Van Huerck, Syn. Diat. Belg., pl. 77, f. 2 (Anvers 1882) ; Van Huerck, Treat. Diat., trans. Baxter, text-fig. 122 (London 1896) ; H. & M. Peragallo, Diat. Mar. France, 242, pl. 54, f. 9, pl. 55, f. 5–6 (Paris 1899).

This species is closely related to *C. biangulatus* Grev., *C. Lorenzianus* Grun., *C. impressus* Grun. ex A. Schmidt, *C. birostratus* Deby and *C. inconspicuus* Deby. How many specific entities are represented by this list is not a point which we have investigated, but it seems unlikely to be more than two. In all of them the costæ, which are 2–2½ in 10μ at the margin, are more distant than in *C. innominatus* Ross and Abdin, which is also distinguished by the absence

of any short striæ between each alar canal, whereas in all these other forms there are two or three.

C. innominatus Ross and Abdin is apparently a rather uncommon diatom and when it has been met with it has always been called *C. parvulus* W. Smith. As is pointed out above, this is an error for which Grunow (1862) was first responsible. The fact that the species has been known from that date without acquiring a specific name has led us to choose the epithet which we have adopted for it.

At Blakeney Point this diatom has been found in the shingly tributary creek which is also one of the habitats of *Surirella hispida* Ross and Abdin. It would appear that it also is to be classed as mesohalobous and euryhaline.

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DESCRIPTION OF PLATE.

Fig. 1.—*Nitzschia irregularis* Ross and Abdin. (×1500.)

Fig. 2.—*Surirella minima* Ross and Abdin. (×1000.)

Fig. 3.—*Surirella hispida* Ross and Abdin. (×1000.)

Fig. 4.—*Campylodiscus innominatus* Ross and Abdin. This figure has been divided to show two levels of focus. (×1000.)

Photomicrographs by H. Malies, F.R.M.S.

XXI.—THE USE OF THIN SECTIONS OF ENTIRE ORGANS IN 578.65 MORBID ANATOMICAL STUDIES.

By J. Gough, M.D., and J. E. Wentworth.

(Department of Pathology and Bacteriology, Welsh National School of Medicine, Cardiff.)

Our original aim in designing this technique was to obtain representative sections of the entire lung for comparison with radiographs taken during life. The method was first applied to an investigation of respiratory disease in coal-workers, and we have used it principally for research in this disease, but it is applicable to other diseases and to organs other than the lung.

Large-section techniques have previously been used, but they have mainly been intended for microscopic study of large pieces of tissue or entire organs and involved standard methods of staining and mounting. The preparations described here are, however, intended for naked-eye examination and they are in natural colours. One special innovation is the mounting of the sections on paper. This produces striking results in the case of pneumoconiosis and certain other diseases of the lung, but this dry mounting will probably have a smaller field of application than mounting the sections in fluid in thin cases of Perspex.

The organs are fixed in a formalin solution. The formalin is subsequently washed out of a representative slice of the tissue and the latter is embedded in gelatin. At first we divided a slice of lung into six or eight blocks and, after cutting each separately, pieced the sections together as in a jigsaw, mounting them on glass. A Cambridge sliding microtome was used for cutting. The maximum size which could be cut with this machine in its original form was about 3 × 2 inches. The machine was then modified and adapted to take larger blocks and it was found possible to cut sections up to 10 × 8¾ inches. Recently the Measuring and Scientific Equipment Company have made us a special microtome for the purpose and it is proving very satisfactory.

During the early stages of the development of the technique we met trouble with the gelatin. We had difficulty in obtaining suitable gelatin, but the supply position improved and we found that Nelson's No. 1 serves our purpose. We also had the problem of preventing the gelatin being decomposed by contaminating bacteria when it was kept at 37° C. But in respect of this difficulty we had a piece of good fortune. Cultures of the gelatin showed gram-negative bacilli, including *Pseudomonas pyocyanea*. One of us had particular experience of this organism as a contaminant of wounds and, based on the results obtained with the latter, we added to the gelatin 2 p.c. phenoxetol, which we expected to be effective. Not only did it keep the gelatin sterile, but it unexpectedly restored

the natural colour to blood pigments, and these colours have remained unchanged in dry-mounted specimens during the past two years and it appears they will be permanent.

It was found necessary to observe the standard precautions in making the gelatin solutions. The gelatin is soaked in water for at least an hour and then warmed in a water-bath to dissolve it, but not allowing the solution to boil.

In addition to enzymes from bacteria, proteolytic enzymes of tissue origin caused trouble. Digestion occurred, especially in the lower parts of the lung, where blood had gravitated post mortem. This autolysis was often marked in normal lungs and in those showing pneumonia. Enzymes not only digested the lung but also the gelatin after embedding. Long fixation for weeks or months in the formalin seemed to destroy the enzymes and the subsequent digestion of the gelatin was lessened or obviated.

Our first preparations were mounted on glass, but having lost one or two valuable specimens due to accidental breakage we tried other sorts of mounting. Old X-ray films with the emulsion removed were only fairly satisfactory, as they became wrinkled, but a method was evolved of mounting the sections on paper. During an examination of the possibility of mounting the sections on Perspex it was found that if a wet section was placed on Perspex which had been flooded with a mixture of glycerin and gelatin and then covered with paper, that after thorough drying the paper, with section firmly attached, could be stripped off the Perspex. The preparations have a glossy surface. Various types of paper have been tried, but ordinary filter-paper is best. We use Whatman's No. 1.

The paper-mounted sections may be kept in book form, and we use this method for routine storage. For display the sections are best mounted between two thin sheets of Perspex or glass and illuminated by transmitted light. For this purpose X-ray viewing boxes are admirable.

The sections are cut at 500μ and from a block of tissue a dozen or more sections can usually be obtained. The sections have a special value in the convenience with which they can be sent by post, and we have been able to provide many centres at home and abroad with representative examples of particular forms of disease. The preparations are thus of value for disseminating information for teaching and research purposes.

Lungs showing pneumoconiosis give striking results because there is plenty of contrast in colour and in density of tissue between normal lung and emphysema.

For other organs such as the brain and liver the sections have been mounted in thin Perspex cases in the usual mounting fluids. By using different coloured Perspex as backgrounds, especially black and cream, certain features can be made more prominent than in whole specimens. We have as yet not applied the method very widely, except in the case of the lung, but it holds out promise in other directions.

TECHNIQUE.

The lung was removed whole without rupturing the pleura. If there were dense adhesions the parietal pleura was taken out together with the lung. (A

few small tears did not matter greatly, except when there were large emphyse-
matous bullæ.) It was then cut off at the hilum and fully distended by running
into the major bronchi

Liq. formaldehyde (40 p.c.)	500 ml.
Sodium acetate	200 gm.
Water	to 5,000 ml.

by means of a tube and cannula from a reservoir about 4 feet above the lung.
There was no need to tie the bronchus after running in the fixative. The lung
was then placed in a container of fixative large enough for it to float freely
without distortion from pressure. The specimen was covered with a cloth wet
with fixative. The amount necessary to distend the lung was found to vary up
to about 2 litres and in the container was a further 3 litres. It was allowed to
fix for two days or longer, after which a slice about ¾ inch thick was cut. This
could be taken in any direction. A sagittal one proved to be convenient. Good
results could usually be obtained after a few days' fixation, but in the absence
of any urgency the slice was allowed to continue to fix for two months or longer,
to destroy proteolytic enzymes completely. The slice was then washed in
running water for at least 72 hours to remove the formalin.

Following fixation the slice was place in a solution of :

Gelatin	100 gm.
*Propylene phenoxetol	10 ml.
or Phenoxetol	20 ml.
Capryl alcohol	5 ml.
Water	850 ml.

It was necessary to remove air from the slice to assist penetration by the
gelatin. This was done by the usual method of exhausting with a water vacuum
pump. The lung slice was placed in a jar containing the gelatin solution heated
to about 60° C. and put under a bell-jar connected to the vacuum pump. A
solution of agar was found useful as a seal around the jar. With an efficient
glass pump sufficient air was removed within an hour, during which time the
gelatin remained fluid at ordinary room temperature. The specimen in the
same gelatin solution was then placed in an incubator at 37° C. for 72 hours.
While in the oven the temperature of the gelatin should not be allowed to exceed
37° C. as overheating interferes with gelling properties. At this stage the lung
was in a covered container, made from Perspex, in which it could lie flat and be
completely immersed.

Next it was transferred to :

Gelatin	200 gm.
Propylene phenoxetol	10 ml.
or Phenoxetol	20 ml.
Water	800 ml.

and the specimen was left in this a further 72 hours at 37° C., after which it was
transferred to :

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* Obtainable from the Nipa Laboratories Ltd., Treforest, Nr. Cardiff.

Gelatin	..	..	..	..	..	..	250 gm.
Propylene phenoxetol		..	..	..	..	10 ml.
or Phenoxetol	..	..	..	..	..	..	20 ml.
Water	..	..	..	..	..	..	800 ml.

for yet another 72 hours at 37° C. The specimen was then ready to embed, using this same gelatin solution. The gelatin containing the specimen was cast into a block in a rectangular Perspex mould. This had the same surface dimensions as the block holder of the microtome, was 2 inches deep, and had a loose, removable bottom. From 10–50 ml. of liq. formaldehyde (40 p.c.) was mixed with the gelatin in which the specimen was immersed and sufficient of the solution was poured into the mould to cover the bottom. The specimen was then placed in the mould, making quite sure that no air bubbles were trapped under it, and covered with the rest of the gelatin and allowed to set. The amount of strong formalin to be added was learnt by experience. If the gelatin solution before adding the formalin was of a thin, syrupy consistence, 50 ml. of formalin could be added. If it was more viscous less formalin was added. It was found necessary to add the formalin a little at a time, stirring constantly, and as soon as the gelatin began to thicken the addition of formalin was stopped and the lung slice embedded.

The block was cooled thoroughly for several hours (this time could be shortened by placing in a refrigerator). The block was then removed from the mould by pushing out the removable bottom with the block attached and immersed in some of the 10 p.c. formalin fixative, bottom downwards, overnight. The Perspex bottom was removed from the gelatin block and the latter immersed in the formalin for a week to harden.

Before attaching the block to the microtome holder the gelatin was scratched with a wire brush and the upper surface of the block holder warmed with hot water and then molten gelatin (15–20 p.c.) poured on it. The block was then placed on the holder, scratched surface down, and pressed into intimate contact by means of leaden weights and allowed to cool. Cutting was usually possible within an hour. If, on cutting the block, only the upper and outer parts were sufficiently hardened and the deeper layers were still soft, the block, still attached to the holder, was returned to the formalin for a few more days.

The microtome knife needed to be razor sharp, but the presence of small notches did not matter ; honing proved sufficient and stropping was not necessary. The cutting was carried out by a series of short "to and fro" movements. The sections were kept under water until they were mounted. If desired a reserve of unmounted sections may be kept in the formalin fixing solution. They were strong enough to be easily handled.

Mounting on Paper.

The following solution was used :

Gelatin	..	..	..	..	..	..	75 gm.
Glycerin	..	..	..	..	..	..	70 ml.
10 p.c. solution camphor in methylated spirits	..	10 ml.
Water	..	..	..	..	..	..	850 ml.

Some of the warm solution was poured over a sheet of Perspex and after trimming the surplus gelatin round the edges of a section this was placed flat on the Perspex and covered with a sheet of Whatman's No. 1 filter-paper. By running a rubber roller squeegee lightly over it surplus gelatin and air bubbles were squeezed out. The Perspex sheet was then stood on end for 15–30 seconds to drain before being laid flat until the gelatin set. It was hung up at room temperature until there were no obvious damp patches and then placed at 37° C. overnight to dry completely. The paper, with the section mounted on it under a glazed surface, could then be easily peeled off the Perspex. It was found to be important to wait until the specimen was quite dry and peeled off easily before attempting to remove it from the Perspex. After trimming the edges of the paper the specimen was kept in an envelope to prevent it from curling, before transferring to book or Perspex mounting.

Mounting in Fluid.

Sections which have exudates or other features likely to be spoilt by drying should be mounted in thin Perspex cases, using the ordinary mounting media.

SUMMARY.

A technique is described for cutting sections of entire organs. These sections have been mounted dry on paper and examined as transparencies. They may also be mounted in fluid in thin Perspex cases.

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681.2   XXII.—A SIMPLE ANTI-VIBRATION DEVICE.

By A. E. J. VICKERS.

ONE PLATE.

WORKING in a laboratory subjected to much vibration from road traffic as well as from railway shunting operations and other industrial vibrations led to a trial of numerous methods for preventing the transmission of these vibrations to photomicrographic apparatus, delicate chemical balances, and optical and electrical apparatus. Of all systems tried, the best and cheapest has been found to be that illustrated in the photographs. The anti-vibration unit consists of a piece of 6-inch cast iron gas pipe about 5 cm. deep; it is convenient but not essential to mount this on a simple wooden base. The piece of pipe serves to constrain the outwards movement of three tennis balls (or sorbo rubber balls) placed within it; the three balls form a base to support a fourth ball placed upon them and forming the apex of a triangular pyramid. As many of these units as are necessary to support a piece of apparatus are used, usually three or four units are required. The photograph shows a balance mounted upon four such units. In this case the device is so effective that a cement testing machine operating upon the same bench does not interfere with the use of the balance. A photomicrographic apparatus used on a light floor at the top of a building subject to vibration has been found to show no effects of vibration.

No originality is claimed for the idea; the note is intended to bring it to the attention of microscopists as a cheap, well tried, and successful method of dealing with vibration.
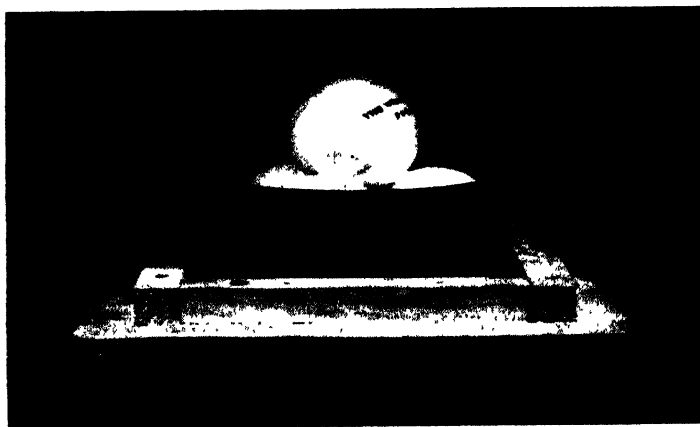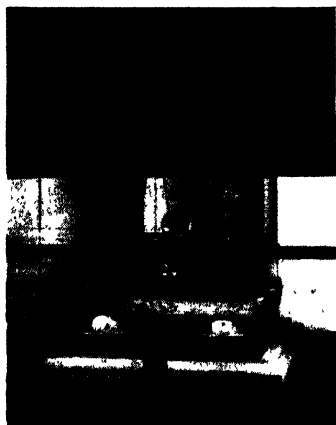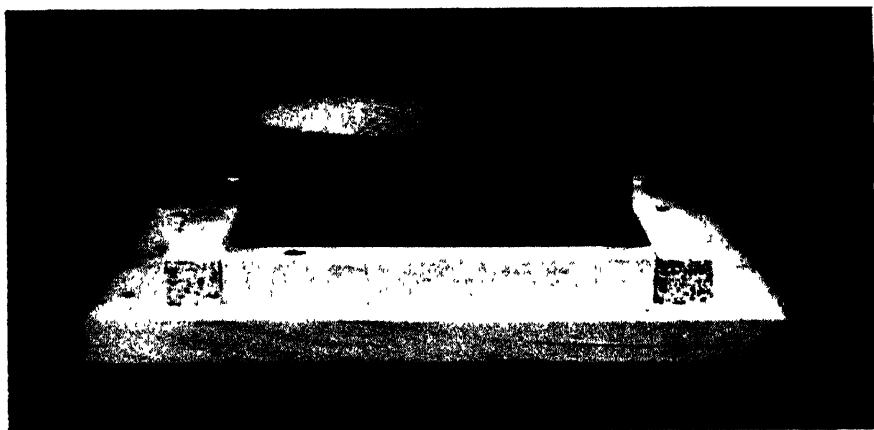
XXIII.—THE GALILEO PHASE-CONTRAST MICROSCOPE. 535.822

By R. H. Powell.

The instrument here set up is the Phase-Contrast Microscope made by Messrs. Officine Galileo, Milan, Italy. It is believed to be the first microscope designed purely for phase-contrast work. Its distinguishing features are :

(1) The use of a substage which is attached to and readily removable from a focusing dovetailed slide by means of a spring-loaded pin, which engages a corresponding tapered slot in a tongue on the substage assembly. The substage assembly can, therefore, be removed easily and rapidly, leaving the focusing device *in situ*. The illuminant is built on to the substage and consists of an 8-volt 0·6-amp. M.E.S. lamp in a centring fitting. The apex of the bulb is frosted and the lamp-house tube is provided with slots to accommodate yellow, green, and blue filters and a matt diffusing screen. The beam emergent from the lamp-house condenser passes through an iris diaphragm and thence through one of three apertures in a disc provided with a milled edge. Aperture No. 0 is for direct observation ; No. 1 is the condenser annulus for phase-contrast work ; No. 2 is a diaphragm which produces in the eye-piece an image of the same overall intensity of illumination as when aperture No. 1 is used. The beam then passes through a totally reflecting prism into the Panchratic condenser whose aperture is adjustable from 0·16 to 1·4. The condenser can be centred by means of the centring screws provided.

(2) The microscope body is provided with a lens which can be swivelled into and out of the optical path and can be used to focus the image of the phase rings in the objective. This device greatly simplifies the centring of the whole system for phase-contrast work. In other microscopes it is necessary to remove the eye-piece, to substitute a telescope, to focus the image of the phase rings, and then to centre the phase-contrast system. The auxiliary telescope is then removed, the normal eye-piece substituted, and this procedure must be repeated with each change of objective. In the Galileo instrument the body tube lens is built into the body and is merely swivelled into or out of the path as required. The centring of the system is, therefore, a matter of the utmost simplicity. This will be evident when the microscope is closely inspected.

(3) The swivelling binocular head is a feature which commends itself, particularly where an instrument has been set up by one party and another party wishes to examine the specimen.

The steps to be taken to put the instrument into adjustment for phase-contrast work are as follows :

(1) Switch on light source.

(2) Set condenser annulus to No. 0 and roughly focus specimen.

(3) Swing in auxiliary lens and focus a sharp image of the objective phase ring.

(4) Close down iris diaphragm.

(5) Focus Panchratic condenser until the image of the diaphragm is about the same size as the inside of the image of the phase ring.

(6) Centre up this image by the use of the condenser centring screws.

(7) Open iris diaphragm and rotate condenser annulus to No. 1 position.

(8) Centre condenser annulus by means of condenser annulus centring screws, so that its image is concentric with the image of the phase rings.

(9) Adjust the Panchratic condenser until the image of the condenser annulus is exactly coincident with the image of the phase rings.

(10) Swing out the auxiliary lens and the microscope is now adjusted for observation under phase-contrast conditions.

The specimen shown on the microscope is a cell in the outgrowth of tissue culture of the lung of a newt (*Tritunus cristatus*). This magnification is × 400 and the specimen displays fat cells and three or four nucleoli in the nucleus.

A Board of Trade licence needs to be secured before the instrument can be imported from Italy.

XXIV.—*HÆMOPROTEUS* SP. INVESTIGATED IN FALCONS   **576.893**
(*FALCO TINNUNCULUS RUFICOLIFORMIS* AND *FALCO NAUMANNI PEKINENSIS*) OF EGYPT.

By M. H. Haiba, M.V.Sc. (Zoology).

(School of Veterinary Medicine, Fouad I University, Giza, Egypt.)

Two Plates.

Avian hæmosporidia have recently become of interest to scientists in view of the fact that they prove of real value in laboratory studies, where a large amount of experimental material can be readily made available since the incidence of infection is usually high. In non-fatal cases the course of infection can be followed for years or even during the whole life of the bird. The recent propagation of the parasites on a large scale in tropical countries has helped tremendously in obtaining fuller knowledge regarding certain phases which had hitherto remained obscure. They also provide an excellent experimental field to test the activity of the newer synthetic compounds which possess certain well defined pharmacological characteristics that are not found in the already known remedies.

In 1946 the author studied various hæmosporidia parasitic in Egyptian birds ; he is convinced that further work in this field would be highly instructive.

A case of acute hæmoproteus infection came to the author's notice on May 20th, 1948, in a young female falcon (*Falco tinnunculus ruficoliformis*, also seen in *Falco naumanni pekinensis*) which had been examined for plasmodial parasites. Although falcons abound in Egypt, they do not seem to have been investigated for this parasite and references to the subject as it affects Egypt are almost non-existent.

Infection with parasites of the genus *Hœmoproteus* has been described in more than 100 European species of birds of all families in all continents ; but most of these publications are limited to description of the growing, typically elongated parasites inside the red blood corpuscles. During the present investigation, various stages and forms of the parasite were accidentally encountered.

In Giemsa-stained peripheral blood preparations the sexual forms were seen in various peculiar forms and stages of maturity. The smallest stage observed in red blood corpuscles was 0.5–1.0μ ; these faintly sky-blue cytoplasmic structures were occasionally found free or attached to the host cell before entrance. This is in contradistinction to Schaudinn's supposition (1916) that *Hœmoproteus* parasites would only attach themselves to the cells. In the same year Argutinsky also agreed that malarial parasites seen in profile were attached to and not inside the cells.

Following this step by step, bigger forms were never seen attached to the margin of blood cells. Evidence of their further increasing growth, their position in relation to the cell nucleus, and the destruction of the host cell itself are definitely in favour of intracellular development. They started as delicate irregular dots or rings whose margin was light blue with one or more reddish chromatin nuclei; the centre, however, was colourless (fig. 1). The nuclei were not always found in the same situation in the parasite, but were sometimes seen in protrusions in the form of points, rods, or ring segments (fig. 2, A). These earlier stages were usually noted to be either at the poles or at the sides of the cell nucleus (fig. 1 and fig. 6, A), thus confirming Manwell's observation (1935). The red cells parasitized by this stage were unaltered and their nuclei, even in cases of multiple infection, not displaced (fig. 2, A). No pigment granules were noted in this stage of the parasite. Slightly bigger forms, about 3–5μ, oval or elongated in shape, with two to four very fine refractile, dust-like, pale-brown pigment granules were sometimes seen (fig. 3, A). In some preparations multiple infection of the red cells with this form was seen, though not as often as single infection. The presence of one parasite did not hinder the invasion of others, since infection with two or more parasites occurred without typical arrangement to one another or to the host cell nucleus which was sometimes seen slightly displaced (fig. 3, B, C, and D). In such cases the limits of the parasites were not clearly traced and their cytoplasm appeared confluent, giving the impression of being one parasite (fig. 2, B and D). Sex could not be differentiated before the small gametocytes reached about the size of the red cell nucleus, 6–7μ, when their nuclei and cytoplasmic net-work became clearly differentiated. This confirms Mayer's statement (1911) regarding *Hæmoproteus synii*. With increase in size, the foamy cytoplasm of the mature female forms appeared darker, denser, and with narrower meshes; the nucleus was also denser and more compact (fig. 5). In the male (fig. 2, C) the cytoplasm was faintly stained and composed of diffuse nuclear constituents filling the greater part of the parasite. Pigment granules started separately in the meshes of the cytoplasm and gradually collected in one vacuole. Those of the microgametocytes were fine, 12–14 in number, and mostly grouped in one or in both ends of the dumb-bell-shaped parasites, while the nucleus filled up the middle area (fig. 8, A and fig. 2, C). Some of these granules were occasionally found centrally at the top of the nucleus (fig. 4, D). In macrogametocytes the granules were relatively bigger, 16–18 or more in number and irregularly scattered all over the cytoplasm (fig. 5, A), but occasionally grouped asymmetrically in a bigger clump at one end (fig. 4, A). The growing mature individuals gradually reached their various characteristic forms depending on their first enplacement inside the parasitized cells. Those gametocytes whose young stages began at the longer side of the red cell nucleus mostly exhibited the familiar elongated dumb-bell shape with smooth, rounded ends, assuming three forms: in the first form, they extend unilaterally, nearly straight along the red cell nucleus to the margin of the cell cytoplasm. They measure 8–10 × 3–3·5μ and neither alter the shape of the cell nor displace its nucleus (fig. 2, C); occasionally, however, cases were seen with slight or complete displacement of the nucleus (fig. 4, A). In the second form, the gametocytes grew towards the nucleus by surrounding it with one or both

Fig. 1

Fig. 2
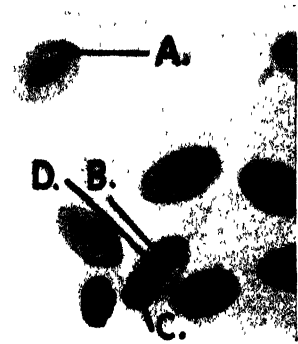
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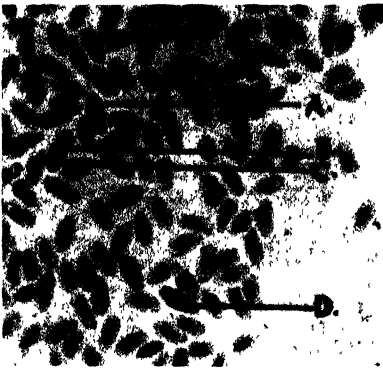
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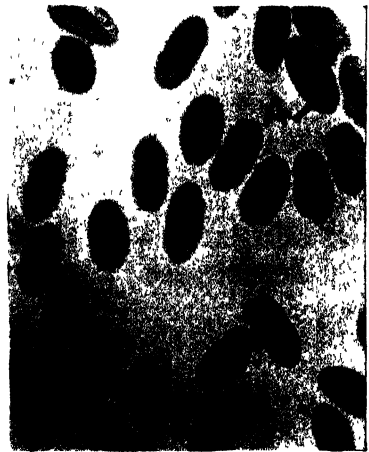
Fig. 5

Fig. 6
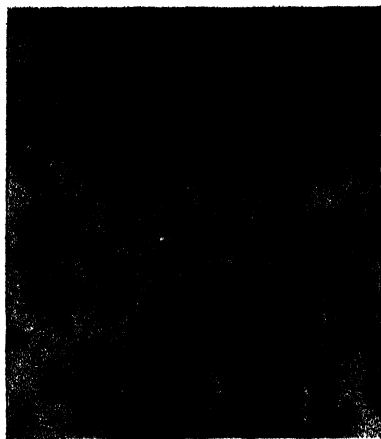
Fig. 7
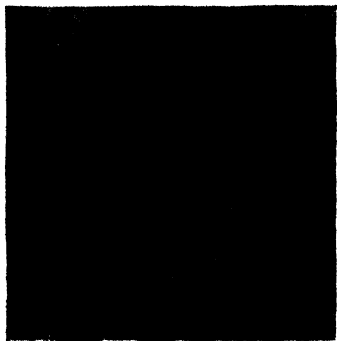
Fig. 8

Fig. 9

Fig. 10
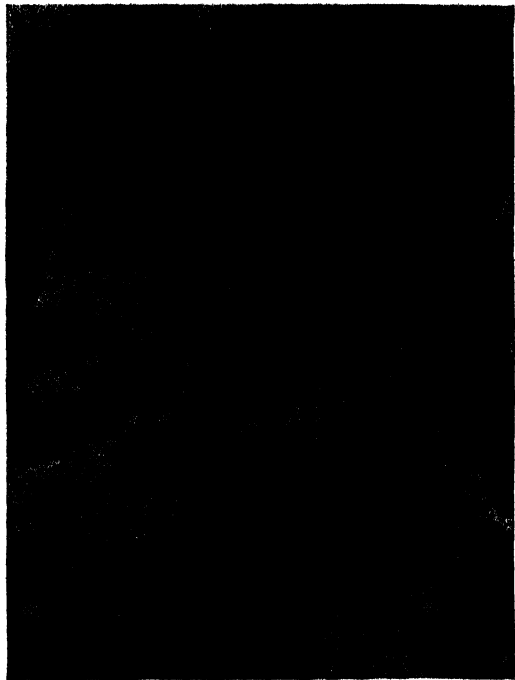
Fig. 11

ends, causing complete or little displacement (fig. 5, A, and fig. 6, B), but there were cases in which the parasite surrounded the nucleus in a complete ring, filling the parasitized red cell completely (fig. 6, C). In the third form, which measured about $12–15 \times 4–5\mu$, they grew away from the cell nucleus, completely displacing it with alteration of the parasitized red cell (fig. 7, A). In cases of more polar position (fig. 2, B, and fig. 3, C), as Wasielewskii and Wülker stated (1918), the mature gametocytes were seen placed like a cap on the pole of the nucleus which was slightly displaced (fig. 8, B). Occasionally, round or spheroidal parasites of this type varying from $6·8–10·2\mu$ were observed filling the whole red cell so completely as to push its nucleus to the extreme end (fig. 9, A), or even entirely outside. Some parasitized red cells were so packed with two spheroidal gametocytes as to bring their nucleus to a transverse position (fig. 4, B and C). Very rarely the author observed monocytes harbouring spheroidal mature gametocytes and being ruptured by the parasites (fig. 10, A).

In addition to the sexual stages investigated in the peripheral blood, the agamogony stages of the parasite originating in the reticulo-endothelial system of internal organs of killed or naturally dead falcons were also observed. Tissues from various internal organs obtained at autopsy were fixed in Zenker's fluid for 24 hours. Reginald Hewitt's device (1939) for staining sections was adopted throughout the whole work because it gave the most satisfactory results in differentiating between the parasites and the host cells. The findings of Aragão (1907–8) and Anschutz (1909), Negri (1913), Acton and Knowles (1914), and Gonder (1915) made it probable that schizogony would be found in the endothelial cells of the blood vessels of various internal organs, particularly the lungs, spleen, liver, bone marrow, and brain and also in mononuclear leucocytes. It was found that in heavily infected old falcons none of the stages was seen in smears of organs; but in young acutely infected birds whose peripheral blood contained occasional young gametocytes, numerous schizont stages were found in the brain (fig. 11) the liver, and the spleen; they were rare in the lungs and kidneys. In successful preparations small or bigger blue-stained spherical, amœboid or sausage-shaped schizonts in various stages of maturity were noted regularly in the cells lining the capillaries. Each was seen to contain a single big nucleus or numerous small faintly red irregular nuclei, from 2 to 30 in number. Free parasites of similar forms were also seen, mostly in the brain within the lumen of its capillaries. The author is of opinion that there is sexual differentiation among these schizonts, as in gametocytes, since they appear to take the stain differently. A few stained faintly and contained fewer small daughter nuclei, 2 to 6 (fig. 6, B). Wasielewskii also suggested (1918) that these are male schizonts. The others, which were the majority, were bigger in size, deeply stained, and contained a greater number of nuclear divisions, accordingly they may be considered as females (fig. 11, A).

It appears from this study that the developmental stages of hæmoproteus of falcons differ from those of hæmoproteus infecting other birds studied by the author, in respect to their forms and position.

ACKNOWLEDGEMENTS.

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OBITUARY.

JOSEPH AUGUSTIN CUSHMAN.

(January 31, 1881–April 16, 1949.)

WITH the death of J. A. Cushman, and the closing down of his Laboratory for Foraminiferal Research at Sharon, Mass., what will always be known as the " Cushman Era " comes to an end. His " Foraminifera, their Classification and Economic Use," first published in 1928 and now in its fourth edition (1948), based on phylogenetic discoveries and theories, has revolutionized the study of the group and practically superseded earlier artificial classifications. Whatever modifications future research may bring about, this work will always be a landmark in the study of the group.

J. A. Cushman came of old New England stock. He told me once with pride that his ancestors went to America with the Pilgrim Fathers. Born at Bridgewater, Mass., in 1881, he graduated at Harvard in 1903 and completed his studies at Cambridge, Mass., with a Ph.D. in 1909. He was Museum Director of the Boston Society of Natural History from 1913 to 1923.

He first became interested in Foraminifera through working at the Woods Hole Laboratory of the U.S. Fish Commission, and followed it up by a study of " Albatross " material at Washington. Later he became connected with the U.S. Geological Survey, formed his ideas of the importance of Foraminifera to petroleum discovery in the correlation of strata, and undertook commercial geological work with various oil companies.

To publicize his views he started the Cushman Laboratory for Foraminiferal Research at Sharon, Mass., in 1923 and in 1925 began the issue of a quarterly publication " Contributions from the Cushman Laboratory for Foraminiferal Research " which is now in its twenty-fifth volume. Besides the " Contributions," the laboratory has also issued twenty-four special publications of importance, including the celebrated " Foraminifera—their Classification and Economic Use." He made two trips to Europe, in 1927 and 1932, to study types and collect material from type localities. With all this he retained his connection with the U.S. Geological Survey, was appointed Geologist to the Survey in 1926, and retained that post until his death.

Some idea of Cushman's terrific energy may be grasped from the fact that, in addition to the conduct of the Laboratory and the training of students from many countries, he and his collaborators have published over 500 papers and reports of varying sizes. Several more are to be published posthumously. He also gathered together at the Laboratory one of the most complete libraries of foraminiferal literature in the world, a card catalogue of nearly 95,000 references

and figures, and a collection of slides of which over 62,000 have been catalogued. Uncatalogued slides are even more numerous.

He bequeathed all his collections and material to the Smithsonian, and they will be transferred to the U.S. National Museum at Washington for preservation and for the future use of students.   The twenty-fifth anniversary of the laboratory was celebrated in 1948 by the presentation to Cushman of an album of letters received from correspondents and workers all over the world.   He was then very ill, but he told me of the very great pleasure it gave him.   The Laboratory is now closing down after completion of the work in hand.

Cushman was connected with many societies, and among the honours bestowed on him are an Honorary D.Sc. conferred by Harvard in 1937 ; the Hayden Memorial Gold Medal awarded by the Philadelphia Academy of Natural Sciences in 1945 ; and the Honorary Fellowship of the Royal Microscopical Society in 1938.

In 1912 the late Sir John Murray, recently returned from the " Michael Sars " trans-Atlantic expedition, asked me at a meeting of the " Challenger Society " what I knew of a man named Cushman.   I had to reply, " Nothing— not even the name."   Murray said that I had better keep my eyes open, for he was making quite a stir in America.   His papers and correspondence soon made his name familiar, but I did not meet him personally until he came to Europe in 1927, when I saw him by appointment at the British Museum, where he wished to examine our collections.   I took an immediate liking to the tall and distinguished man whom I met, and from then on we were on the best of terms, in spite of many differences of opinion ; our correspondence continued up to his death.   In his last letter, written just a month before his death, he referred to the pleasure which our correspondence had given him.   A. E.

ABSTRACTS.

MICROSCOPES AND MICROSCOPY.

A New Ultra-violet Microscope.—E. H. LAND, E. R. BLOUT, D. S. GREY, M. S. FLOWER, H. HUSEK, R. C. JONES, C. H. MATZ, and D. P. MERRILL (" A color translating Ultra-violet Microscope," *Science*, 1949, **109**, 371–4; 23 refs., 3 figs.). The instrument employs a superhigh pressure, water-cooled mercury arc as a light source, a Wadsworth-type grating monochrometer, a microscope using apochromatic objective lenses and identical lenses as condensers ; the camera takes 35-mm. motion-picture film. There is also a film-processing station, a three-beam projector, and the viewing screen. The microscope is not only achromatic but apochromatic from 220 to 800 mμ, and by using simultaneously or sequentially three different ultra-violet wavelengths it is possible to convert the ultra-violet images so obtained into visible images in three primary colours. When these three images are superposed a visible image in full colour is obtained.   G. M. F.

Ultra-violet Microscopy.—B. K. JOHNSON (" A Compound Reflecting Microscope of High Aperture for use in Ultra-violet Light," *J. Scient. Instrum.*, **26**, No. 5, 148–50). The use of the high-power reflecting microscope enables systems to be designed in which the focus is constant for both visible light and in the ultra-violet. Opaque objects can be examined by vertical illumination without the production of reflections from the polished surfaces of the objective.

Compound microscope systems are described which have been developed from some of the author's earlier designs. The first magnification is produced by reflection from the back surface of a lithium fluoride meniscus lens, followed by a further dioptric magnification through fused quartz hemispherical lenses and a Ramsden-form eye-piece corrected for ultra-violet light. Spherical surfaces are employed throughout, and numerical apertures up to 1·27 can be obtained without obstruction to the image-forming beam. Specifications are given and the residual aberrations are shown graphically and tabulated.   B. O. P.

Microscopy and Nuclear Physics.—H. WAMBACHER (" Mikroskopie and Kernphysik," *Mikroskopie*, 1949, **4**, 92–110). The elementary theory of the structure of the atom is outlined and the main types of nuclear reactions are then described. An account is given of the part played by the microscope in the various methods by which atomic particles are detected. Scintillation methods employ a microscope for determining the number of flashes produced on a fluorescent screen by the impact of particles, and photometric comparison microscopes are used to compare the activity of two radioactive sources. The results obtained by electrical and scintillation methods are displayed graphically and a short account is given of the determination of mass, using a microscope to measure the deflection on a fluorescent screen produced by the

passage of particles through a magnetic field. Photographic methods are then considered. Diagrams and actual photomicrographs are used to illustrate the paths of α particles and protons in the emulsion and examples are given of various types of nuclear reaction as revealed by tracks on a photographic plate. Applications are discussed and the measurement of the tracks is described. Finally, methods of estimating the size of the earth, based on observations on decolorized halos in mica and other minerals, are briefly surveyed. B. O. P.

Micro-Hardness Testing.—H. BÜCKLE (" Ein Nomogramm für die Mikrohärteprüfung," *Mikroskopie*, 1949, **4**, 183–6). The formula $H = 1854P/d^2$ Kg./mm.^{2} relates the Vickers Hardness Numeral H to the applied load P in grammes and the length of the diagonal of the impression, d, in microns. From this is derived the relationship $\dfrac{\log H_2 - \log H_1}{\log B_1 - \log B_2} = 2$, where B_1 and B_2 are the eye-piece scale readings corresponding to the hardness numerals H_1 and H_2, for a given load. This enables nomograms to be constructed in the form of parallel logarithmic scales of B, H, and the load from which hardness figures can be read for any combination of impression size and load. B. O. P.

New Light Sources for the Microscope.—F. DESVIGNES (" Les nouvelles sources de lumière utilisabiles en microscopie, "*Microscopie*, 1949, **1**, 132–40; 12 figs.). In this review are discussed tungsten and arc lamps, the concentrated arc, and high-pressure mercury vapour lamps. The characteristics of each type of lamp are fully described, especially in regard to luminosity, uniformity, and maintenance. G. M. F.

Phase Contrast.—M. FRANÇON (" Le contraste de phase," *Microscopie*, 1949, **1**, 117–31; 15 refs., 15 figs.). The general principles of phase-contrast microscopy are fully discussed. Contrast in the image, the sensitivity of the method, and other comparable techniques are examined. The best methods and the steps necessary to vary the characteristics of the phase plate are enumerated. G. M. F.

An Infra-red Microscope.—T. STUART-BLACK-KELLY (" A low power Infrared Microscope," *Brit. J. Ophthal.*, **32**, 396; 2 refs.). The basis of the device is a German image converter tube that has the property of transforming an image in the infra-red region of the spectrum (at about 10,000Å.) into a visible image on a fluorescent screen. The equivalent British instrument is now available and the image converter tubes have been produced commercially. The Admiralty are prepared to receive enquiries regarding the assembly of complete microscopes. Any such enquiries should be addressed to the Director of Research Programmes and Planning, Admiralty, Fanum House, Leicester Square, London, W.C.2. G. M. F.

ELECTRON MICROSCOPY.

Summarized Proceedings of Electron Microscopy Conference, Cambridge, September 1948.—V. E. COSSLETT (" Electron Microscopy Conference," *Nature*, 1949, **163**, 32; 10 refs.). R. R.

The Electron Microscope.—A. D. MERRIMAN (" The Electron Microscope," *Metallurgia*, 1949, **39**, 231). A review. V. E. C.

Electron Microscopy.—C. J. BURTON ("Electron Microscopy," *Anal. Chem.*, 1949, **21**, 1). An extensive review. V. E. C.

Spherical Aberration.—P. HUBERT (" Lentille électronique corrigée de l'aberration de sphéricité," *C. R. Acad. Sci. Paris*, 1949, **228**, 233). It is shown on the basis of an approximate expression that a correction of the second order spherical aberration of electron lenses can be achieved by the use of the image force exerted on electrons passing very near the wall of a fine canal. For a focal length of the main lens $f_0 = 0\cdot1$ cm. with a spherical aberration constant $C = 2$ cm. and an acceleration voltage $\phi = 50$ kV., the length of the canal has to be $1 = 0\cdot7$ cm. for a canal radius $r_0 = 10^{-3}$ cm., but the remaining aberrations limit the utilizable angle. A better compromise, leading to partial correction, yields $l = 5$ cm. and $r_0 = 1\cdot6 \times 10^{-3}$ cm. This should almost double the theoretical resolving power of the assumed lens. G. L.

Asymmetry in Electrostatic Lenses.—F. BERTEIN and E. REGENSTREIF (" Emploi des rayons marginaux dans l'étude des dissymétries chez les lentilles électrostatiques," *C. R. Acad. Sci. Paris*, 1949, **228**, 1854–6). If asymmetries are present in an electrostatic lens illuminated by an electron bundle of very wide aperture, a brightened area bounded by hypocycloidal arcs appears superimposed on the image produced by the lens. The type and relative strength of the asymmetries can be judged from the appearance of the figure. In the case of ellipticity of the central electrode giving rise to astigmatism, four hypocycloidal arcs are present. This test of the perfection of electrostatic electron lenses is very sensitive. G. L.

Elliptical Electrostatic Lens.—M. COTTÉ (Potentiel et champ d'une électrode plane percée d'un trou elliptique," *C. R. Acad. Sci. Paris*, 1949, **228**, 377). An exact solution for the field distribution in an electrostatic lens with elliptical hole in the central electrode is given, based on the known solution for the movement of an elliptic disc in a perfect liquid. The axial field distribution can be expressed through the (tabulated) elliptic integral of the second kind. The rigorous solution is approximated near the axis by the solution used previously by Bertein:

$$\phi(z, r, \theta) = \phi_\circ (z) + r^2 [\phi_2(z) + \psi_2(z) \cos 2\theta],$$

where $\psi_2/\phi_2 = R\eta /(z^2 + R^2)$, η being the eccentricity and R the mean radius of the hole. G. L.

Electron Lens with Curvilinear Axis.—P. HUBERT (" Lentille magnétique à axe curviligne," *C. R. Acad. Sci. Paris*, 1949, **228**, 302). General differential equations (to be first-order approximation) are derived for the movement of an electron in a plane magnetic field of a strength varying in a radial direction. It is shown that the variables are separable and that the system can be considered as an arrangement of two crossed cylindrical lenses. The magnetic field distribution can be chosen so that the two cylindrical lenses have a common focus; in this case the system acts as a magnetic lens with a curved axis, which coincides with a principal trajectory. G. L.

Electrostatic Lenses.—MAURICE DUCHESNE (" Sur le calcul dans un objectif à immersion à symétrie axiale du potentiel le long de l'axe et de ses dérivées," *C. r. Acad. Sci.* (Paris), 1949, **228**, 1407). The axial potential is calculated by the method of Bertram (*J. appl. Phys.*, 1942, **13**, 496), involving Bessel's functions and assuming a linear variation of potential between the diaphragms of the two electrodes. The system considered is an image converter (" electron telescope "). It is considered

that the derivatives may be thus calculated with greater accuracy than is obtainable experimentally in an electrolytic trough. V. E. C.

Image Production and Resolution of the Electron Microscope from the Wave-mechanical Viewpoint.—W. GLASER (" Bermerkung zu der Arbeit Bildentstehung und Auflosungsvermögen des Electron Microscope vom Standpunkt der Wellemechanik," *Z. Phys.*, 1949, **125**, 451). The formula given in a previous paper (*Z. Phys.*, 1943, **121**, 647) for the combined effect of spherical aberration and diffraction defect should have the factor 0·78 in place of 0·56. V. E. C.

Image Contrast in Electron Microscopy.—J. HILLIER (" Some Remarks on the Image Contrast in Electron Microscopy and the Two Component Objective," *J. Bact.*, 1949, **57**, 313). It is often desirable to use an aperture smaller than the value for optimum resolution to improve the contrast of biological objects of low inherent contrast, for which very high resolution is not required. An actual physical aperture of the required size (10–20 microns) causes trouble in centering and by rapid contamination in the electron beam. An alternative is to use an auxiliary objective lens of long focal length (11 mm.) at 1 to 1 magnification, in which a stop of 50 microns corresponds to one of 5 microns in normal objectives, at the same illuminating angle. Internal structure of bacterial cells is then much more clearly visible in the electron microscope image. V. E. C.

Image Formation.—B. VON BORRIES (" Electron Scattering and Image Formation in the Electron Microscope," *Z. Naturforsch.*, 1949, **4a**, 51). It follows from the intensity distribution of elastically singly scattered electrons that almost all electrons are deflected out of the image-forming beam by a single elastic collision. The " Aufhellungsdicke " (AD) is defined as that mass-thickness of the object which on the average gives rise to a single elastic collision with every electron in the primary beam. Then the fraction $1/e$, or 36·8 p.c., of the electrons emerge from this thickness without having been scattered. The AD is calculated as function of the beam voltage for different object materials; it gives, to a good approximation, the limiting thickness of the object that is still sufficiently clear and well resolved in the electron microscope.

The inelastically singly scattered electrons fall into two categories: the one part is also rather widely scattered and leads to a further reduction in the image-forming beam; the other part is only weakly scattered and remains in the beam, making it broader and heterochromatic. The " Bremsdicke " (BD) is defined as that mass-thickness in the object which on the average will cause single inelastic scattering of every electron. The BD is also evaluated for a number of scattering substances, as a function of the beam voltage. It is of the same order of magnitude as the AD, and largely dependent on the diamagnetic susceptibility. As an example, the intensity distribution in angle and velocity is quantitatively evaluated for a beam after passing through varying thicknesses of object. The conclusions which may be drawn regarding image formation are discussed. V. E. C.

A New Method of Shadowing.—F. HEIMNETS (" Modification of Silica Replica Technique for Study of Biological Membranes and Application of Rotary Condensation in Electron Microscopy," *J. appl. Phys.*, 1949, **20**, 384–8 ; 6 figs.). A direct silica replica is obtained from the chorioallantoic membrane of a chick embryo by evaporating silica at normal incidence, which, for ease of handling, is strengthened by applying several coats of 0·5–4 p.c. collodion solution. It can then be dry stripped with Scotch tape, and the silica is coated with 0·05–0·10 p.c. formvar solution. After transferring

the replica to a grid with the collodion layer uppermost, the latter is dissolved in amyl acetate, leaving a silica replica strengthened with formvar during examination. The specimen can be shadowed before applying the collodion solution. Rotary shadowing, by rotating the specimen while the evaporation is in progress, avoids loss of detail within the shadows and enhances relief. This method of shadowing can be used alone or in conjunction with conventional shadowing. Examples of some results of this method obtained with salt crystals on chorioallantoic membrane and with KCl particles are given. M. M. B.

Fluorescent Screen for Electron Microscope.—K. B. MERLING (" A New Fluorescent Screen for the Electron Microscope," *Nature, Lond.*, 1949, **163**, 541 ; 2 refs., 4 figs.). It is claimed that a Zn/CdS (Ag activated) viewing screen (H. Meyer and G. A. R. Tomes, *Electronic Forum*, 1947, **27**, 5) has greater brightness and fineness of grain than has the usual willemite type. Thus more detail is visible to the eye, focusing is easier, and hence photography of image detail is simplified. Moreover, the use of a defocused condenser lens, with consequent gain in resolution and uniformity of image illumination, is possible. R. R.

Murogel.—W. O. BAKER (" Murogel, a New Macromolecule," *Ind. Eng. Chem.*, 1949, **41**, 511). Some useful techniques for micrography of very small, low contrast objects which tend to agglomerate by sticking to ordinary support films. V. E. C.

Shadowing.—D. B. SCOTT and R. W. G. WYCKOFF (" Metal Shadowing for the Optical Microscopy of certain Tissues," *Amer. J. Clin. Path.*, 1949, **19**, 63). V. E. C.

Microsectioning.—S. B. NEWMAN, E. BORYSKO, and M. SWERDLOW (" NBS Method for Microsectioning," *Nat. Bur. Stand. Tech. Rept.*, 1949, p. 1358). A new means of preparing very thin sections of biological tissue for light microscope or EM study. The specimen is immersed in *n*-butyl methacrylate, which is allowed to polymerize, giving an optically clear embedding material with highly desirable cutting properties. A smooth continuous advance of the specimen toward the knife of a slightly modified conventional microtome is then obtained from the thermal expansion of a metal specimen holder cooled with compressed carbon dioxide. V. E. C.

Aluminium Alloys.—R. CASTAING (" Recherches au microscope électronique sur les précipitations dans les alliages d'aluminium," *C. R. Acad. Sci. Paris*, 1949, **228**, 1341). Satisfactory aluminium oxide replicas have been obtained from alloys of Al-4 p.c. Cu, showing the form and orientation of the precipitate which forms on the (100) face at above 150° C. V. E. C.

Surface Deformation.—J. R. WHITEHEAD (" Metallic Friction and Surface Damage at Light Loads," *Res.*, 1949, **2**, 145). A short account of the deformation and damage produced on an aluminium surface by a sliding steel needle. The results are similar to those found from optical micrographs of sliders loaded with heavy loads. V. E. C.

Dispersion of Clay Particles.—MATHIEU SICAUD and G. LEVAVASSEUR (" Dispersion des suspensions argileuses aux ultrasons. Interprétation des résultats au microscope électronique," *C. R. Acad. Sci. Paris*, 1949, **228**, 393). The degree of dispersion of clay particles suspended in a liquid and subjected to ultrasonic waves was

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studied by electron micrographic and light absorption methods. The degree of dispersion varied with frequency and reached a maximum at 960 kc./s. for kaolinite and at 320 kc./s. for montmorillonite. Particle size measurements indicated that ultrasonic dispersion gave greater uniformity of particle size than did the normal chemical methods. A. E. E.

Structure of Kieselguhr.—E. PERNOUX (" Examen au microscope électronique de kieselguhrs utilisés comme supports de catalyseurs," *C. R. Acad. Sci. Paris*, 1949, **228**, 1646). Silica diatoms from kieselguhr, as used as catalyst supports (Fischer catalysts), were examined, after various treatments, in the electron microscope. The action of acids tended to dissolve the silica and the resulting gel partially blocked up the fine-structure holes. Subsequent treatment with sodium carbonate cleared the holes. These results agreed with deductions from gas-absorption measurements made in a previous paper. A. E. E.

The Electron Microscope and Its Applications in Metallurgy.—J. ROBILLARD (" Le microscope électronique et ses applications en métallurgie," *Microscope*, 1949, **1**, 65–117; 45 figs., 68 refs.). A detailed account of the principles of the electron microscope, with a short description (illustrated) of all the commercially available electron microscopes. V. E. C.

Electron Micrographic Examination of Powders.—L. DELISLE (" A Method of Examination of Sections of Fine Metal Powder Particles with the Electron Microscope," *J. Met.*, 1949, **1**, Metal Transactions Section, 228–32, A.I.M.E. T.P. 2538E). Electron microscopic observation of metal powders dispersed on a supporting film shows only a projection of the particles. A technique for examining sections through powder particles is described. The metal powders were mounted in a water-soluble melamine formaldehyde plastic, " Aerotex M-3." A thick paste made by mixing powder in " Aerotex " was spread in a thin layer on a glass plate and hardened by polymerizing for 2 hours at 140° C. Normal metallographic polishing and etching practice was followed with such preparations, and formvar or parlodion replicas were stripped and shadow-cast. Micrographs of sections through tungsten, carbonyl nickel, and carbonyl iron particles illustrate differences in grain size and structure of the particles. G. L. J. B.

Replica Techniques for Metallography.—R. SEELIGER (" Darstellung und Messung von Oberflächenrauhigkeiten mit dem Durchstrahlungs-Electronenmikroskop —Presentation and Measurement of Surface Irregularities with the Transmission Electron Microscope," *Metalloberfläche*, 1949, **3**, 9–14). In Part I ten types of replica are briefly described and discussed, and are illustrated by typical micrographs. These include six well-known types in which the replica is a single film and four types in which the replica comprises a plastic film applied to one side or other of an aluminium oxide replica, itself obtained either directly from aluminium alloys or indirectly, from sufficiently hard materials, by use of the aluminium-pressing technique. Part II briefly describes the measurement of surface roughness by stereoscopy and by the shadowing (" optical section ") technique originated by the author (see following Abstract). G. L. J. B.

Observation of Roughness by " Optical Section."—R. SEELIGER (" Über Rauhigkeitsmessung mit dem Elektronenmikroskop: Roughness Measurement with the Electron Microscope," *Z. Metallkunde*, 1948, **39**, 170–72). The " optical section "

method has been adapted to the electron microscope. A replica of the surface is coated by the evaporation of iron, in high vacuum, from a wire, in such a way that a sharp-edged " shadow " is cast. Surface relief is indicated on the electron micrograph by the irregularities in the edge of the shadow. Aluminium oxide films form the best replicas for this purpose. G. L. J. B.

The Electron Microscope in Metallurgy.—J. ROBILLARD (" Le microscope électronique en métallurgie," *J. Soc. Ing. Auto.*, 1948, **21**, 213–22; 40 figs.). [In French.] The theoretical limit of the resolving power is calculated to be 2 mμ. Magnetic and electrostatic lens systems are compared and the latter found preferable. The resolving power of the CSF instrument is given as 4 mμ. A summary of well-known replica methods contains figures for the concentration of the solutions used, as well as details of a double replica method developed in the CSF laboratories. Collodion is used as the first replica and silica as the second, obtained by evaporation in vacuo from an electrolytically polished tungsten wire. Chromium shadowing of these replicas is recommended. Electrolytic and cathodic etching are discussed, and numerous micrographs of aluminium, steel, and tungsten surfaces are shown. M. M. B.

Electron Diffraction of AgI.—J. J. TRILLAT and A. LALŒUF (" Étude, par diffraction électronique, des vapeurs d'iodure d'argent," *C. R. Acad. Sci. Paris*, 1949, **228**, 81). The crystalline form of silver iodide condensed on to collodion under various conditions was examined by means of electron diffraction. Besides the hexagonal type of crystal, cubic crystals of a size not corresponding to previously examined compounds were also obtained. The closeness of the hexagonal crystal dimensions to those of ice may be an explanation of the ability of AgI vapour to condense water vapour from a cloud. A. E. E.

Electron Microscopy of Red Cells.—J. W. REBUCK (" A Simple Direct Method for the Electron Microscopy of Peripheral Blood Cells," *Amer. J. clin. Path.*, 1949, **19**, 217–28; 36 refs., 12 figs.). After concentration of the desired cell layer, smears of the material are made on formvar-covered glass slides. The smears are air-dried, immersed in 2 p.c. osmic acid solution for 14·5–23·5 hours, followed by rinsing for from 10 to 35 minutes in distilled water. Mounting is carried out by a direct method so that the resultant specimens are not replicas. The method consists of: (1) selection of a suitable group of cells under the light microscope; (2) placing a specimen screen over the selected group; (3) destruction of the thickened end of the formvar film at the end of the slide with a scalpel, thus loosening the formvar film at one edge; (4) covering the whole formvar film, including the screen-covered areas, with Scotch tape; removal of the Scotch tape and the now adherent formvar film with interposed specimen screen; sharp dissection of the formvar film about the screen circumference and removal of the screen from the Scotch tape. The cells for study are thus trapped between the specimen screen and the formvar film. Micrographs of small lymphocytes show a finer chromatin pattern than that of normal lymphocytes. G. M. F.

Filamentous forms of Newcastle Disease Virus.—F. B. BANG (" Formation of Filamentous Forms of Newcastle Disease Virus in Hypertonic Concentration of Sodium Chloride," *Proc. Soc. exp. Biol. N.Y.*, 1949, **71**, 50–52; 4 figs., 7 refs.). In water or 0·8 p.c. saline the virus is spherical. If the concentration of saline is increased to 2 p.c. or more the virus becomes filamentous: it may be fixed with osmic acid at this

concentration and will then retain its shape when placed in water. No loss of infectivity occurs during these changes in concentration of saline. Electron micro-photographs illustrate these changes.   G. M. F.

Electron Microscopy of the Virus of Eastern Equine Encephalomyelitis.—F. B. BANG and G. O. GEY (" Electron Microscopy of Tissue Cultures infected with the Virus of Eastern Equine Encephalomyelitis," *Proc. Soc. exp. Biol. N.Y.*, 1949, **71**, 78). Chick embryo tissues were cultured for 6 days with one transfer in roller tubes in a medium of chick plasma, serum, and chick embryo extract. Pieces of this tissue were then exposed to a 10^{-2} dilution of the virus. Chromium-shadowed preparations were made. The general appearance is much like that of a smear of ordinary diplococci. Multiplication may take place by simple binary fission.   G. M. F.

E.M. Studies of Virus-Fowl Red Cell Interaction.—I. M. DAWSON and W. J. ELFORD (" E.M. Studies on the Interaction of Certain Viruses with Fowl Red Cell Membranes," *Nature, London.*, 1949, **163**, 63 ; 1 fig., 7 refs.). The adsorption of influenza, Newcastle disease, mumps, and fowl plague viruses on membranes of lysed fowl red cells has been studied. Micrographs show a general random distribution of virus bodies on the cell membrane, with no evident pattern. As more virus is added, a single close matt covers the entire membrane. Virus concentrations can be reliably estimated by counting the number of bodies adsorbed per unit area of membrane.
                                                                                R. R.

Sectioning of Bacterial Cells.—A. HELGE F. LAURELL (" A Method of Sectioning Bacteria *in situ* for Electron-microscopical and Cytochemical Investigations," *Nature, Lond.*, 1949, **163**, 282; 1 fig., 1 ref.). Impression preparations of growing cultures on clean glass slides are coated with a thin Be layer. In stripping off the Be film, frag-ments of torn, i.e. sectioned, cells, in addition to whole ones, adhere to the metal. Further sectioning on the same preparation can be achieved by repeating the Be de-position and stripping. The sections can be treated with enzymes, etc., thus opening up micro-cytochemical studies.   R. R.

Sectioning of Bacterial Cells.—R. F. BAKER and D. C. PEASE (" Sectioning of the Bacterial Cell for the Electron Microscope," *Nature, Lond.*, 1949, **163**, 282, 1 fig., 1 ref.). Fixed and dehydrated cells of *B. megatherium*, after centrifugal concentration, were impregnated with " Parlodion " (collodion), in ether/alcohol. After one day in 12 p.c. Parlodion the preparation was hardened with chloroform and finally impreg-nated with paraffin. $0 \cdot 1 \mu$ sections of the doubly impregnated block were cut. (Ref. D. C. Pease and R. F. Baker, *Proc. Soc. Exp. Biol. & Med. N.Y.*, 1949, **67**, 470). The original collodion in the sections was removed by immersion in $0 \cdot 1$ p.c. collodion/amyl acetate, when a thinner film was formed. Pieces of this are thin enough for use with 50 kV. electrons. Transverse and longitudinal sections of *B. megatherium* are illustrated.   R. R.

Nuclear Membrane Structure. H. G. CALLAN, J. T. RANDALL, and S. G. TOMLIN (" An Electron Microscope Study of the Nuclear Membrane," *Nature, Lond.*, 1949, **163**, 280 ; 3 figs.). The nuclei of amphibian egg cells are easily isolated, drained, and washed to obtain the nuclear membrane. Micrographs reveal a porous sheet, with pores approximately 300 Å diameter in hexagonal array, spaced 800 Å apart, considered to act as mechanical support for a continuous structureless membrane, which pres-umably determines the permeability properties of the membrane.   R. R.

Tissue Cultures Infected with Vaccine Virus.—J. WIRTH and P. ATHANASIU ("Electron Microscopy of Cells from Tissue Cultures infected with Vaccinia Virus," *Proc. Soc. Exp. Biol. N.Y.*, 1949, **70**, 59). Explants from the renal cortex of adult rabbits were used. Cultures were inoculated with a washed suspension of neuro-vaccinia elementary bodies and electron micrographs of individual cells show opaque spherical bodies in the cytoplasm. The authors have failed to observe similar structures in uninfected rabbit kidney tissue cultures and conclude that they represent the virus elementary bodies within the infected cell.   I. M. D.

Enumeration of Virus Particles.—D. GORDON SHARP ("Enumeration of Virus Particles by Electron Microscopy," *Proc. Soc. Exp. Biol. N.Y.*, 1949, **70**, 54).—A specially designed cell, fitting into the rotor of the air-driven ultracentrifuge, has been designed to deposit influenza virus directly on to a glass cover-slip coated with collodion. A uniform distribution of virus particles over the entire surface of the electron microscope mount is obtained and the aggregation effects inherent in methods involving the drying down of virus suspensions directly on the film are eliminated. The method can be applied quantitatively, and micrographs are shown of swine influenza virus deposited from successive dilutions of the virus suspension. I. M. D.

Viruses of Chicken Pox and Shingles.—J. L. FARRANT and J. L. O'CONNOR ("Elementary Bodies of Varicella and Herpes Zoster," *Nature, Lond.*, 1949, **163**, 260, 11 refs., 2 figs.). Micrographs of material withdrawn from vesicles in cases of chicken pox and shingles show ellipsoidal bodies of fairly regular size and shape. The profile of the virus particle is roughly circular (about 2400 Å diameter), with the height about 750 Å. Staining the particles with phosphotungstic acid or osmic acid reveals areas of differing densities, suggesting a complex internal structure.   R. R.

Nuclear Threads.—W. G. P. LAMB ("Chromatin Threads from Cell Nuclei," *Nature, Lond.*, 1949, **164**, 109, 4 refs., 2 figs.). Calf thymus tissue was disintegrated in various ways, by grinding with sand, Waring Blendor treatment, and shearing between rotating cylinders. E.M. examination shows that the nuclei are drawn out into threads which break off and which have none of the characteristics of chromosomes. Nuclei treated with ultrasonic waves were torn into irregularly shaped pieces.   R. R.

Smear Preparations for Electron Microscopy.—J. SCHULTZ, R. C. MacDUFFEE, and T. F. ANDERSON ("Smear Preparations for the Electron Microscopy of Animal Chromosomes," *Science*, 1949, **110**, 5). A technique for studying chromosomes on the electron microscope is developed from the normal squash or smear technique used in light microscopy. The method is applied to chromosomes from *Drosophila* salivary gland cells and to human pachytene chromosomes. Comparative micrographs from light and electron microscopes are shown and analysed for the *Drosophila* chromosome.   F. W. C.

Electron Microscopy of Chromosomes.—D. C. PEASE and R. F. BAKER ("Preliminary Investigations of Chromosomes and Genes with the Electron Microscope," *Science*, 1949, **109**, 8). Electron micrographs are shown of sections (thickness about 0·1μ) of chromosomes from the salivary glands of *Drosophila melanogaster*. The authors offer an analysis of the micrographs and conclude "it seems reasonable to believe that the discrete particles we have seen are genes."   F. W. C.

Tobacco Mosaic Virus.—R. C. WILLIAMS and R. L. STEERE (" Electron Micrographic Observations of Tobacco Mosaic Virus in Crude, Undiluted Plant Juice," *Science*, 1949, **109**, No. 2830, 308). Crude juice, expressed from the leaf of an infected plant, was dried on the specimen grid and " shadowed." The micrographs showed the typical threadlike particles collected into bundles. Light washing with distilled water before shadowing eliminated the bundles and led to the typical micrographs in which each particle is in contact with the supporting film.   F. W. C.

Nerve Structures.—F. O. SCHMITT and E. DE ROBERTIS (" Electron Microscope Observations of Nerve Structure," *Fed. Proc.*, 1948, **7**, 109). Formol-fixed material, macerated, shows that the nerves have dense edges staining intensely with phosphotungstic acid, a core of relatively low density, and an axial periodicity in the form of transverse bands. In human sympathetic nerves the average width of the bands is about 600 Å and the axial period 650 Å. Three intra-period bands of characteristic density and position have been observed. A dense amorphous material associated with the structures laterally is removable by water extraction. Fibrous axonic structures may be cylindrical or tubular in nature and of somewhat indefinite length, probably many microns.   G. M. F.

The Endospore of Bacillus Megatherium.—G. KNAYSI and J. HILLIER (" Preliminary observations on the germination of the endospore in *Bacillus megatherium* and the structure of the spore coat," *J. Bact.*, 1949, **57**, 23). The endospore is oval in shape, and on germination it sheds a single, highly elastic, thin, perishable coat, resembling the outer coat observed by the same authors in *Bacillus mycoides*.
                                                                        V. E. C.

Study of Teeth.—D. B. SCOTT, H. KAPLAN, and R. W. G. WYCKOFF ("Replica Studies of Changes in Tooth Surfaces with Age," *J. dent. Res.*, 1949, **28**, 31). A description is given of structural details on the surfaces of unerupted and recently erupted teeth as visualized on metal-shadowed collodion replicas. The changes in these details that occur with advancing age are demonstrated through tabular and descriptive data.
                                                                        V. E. C.

Alkali Soaps and Electron Microscopy.—G. S. HATTIANGDI and M. SWERDOW (" Characterization of Alkali Soaps by Electron Microscopy," *J. Res. Nat. Bur. Stand.*, 1949, **42**, 343). The morphological differences between several closely related alkali soaps have been determined by the techniques of electron microscopy. The crystalline alkali soaps consist of an interlocked mesh of bundled fibres, whereas the liquid-crystalline soaps exhibit a flagellar and/or featureless phase. The micellar groupings for the individual soaps are unique, and a scheme based upon electron micrographs is suggested for their characterization. The structures of lithium, sodium, and potassium palmitates are closely related. The laurate, palmitate, and stearate of sodium also exhibit homologous structures. The results of these morphological investigations offer a rapid and accurate means of identification and have been successfully applied to the problem of characterizing commercial soaps of unknown composition.
                                                                        V. E. C.

The Method of Replicas.—A. CLAUDE (" Electron Microscope Studies of Cells by the Method of Replicas," *J. exp. Med.*, 1949, **89**, 425). The formvar replica method has been applied to the study with the electromicroscope of blood cells and bacteria.

The results indicate that the method can reveal details of intracellular structure. Nuclei can be perceived, and also cytoplasmic bodies such as mitochondria and vacuoles.   V. E. C.

The Electron Microscope and the Diagnosis of Smallpox.—C. E. VAN ROOYEN and G. D. SCOTT (" Smallpox Diagnosis with Special Reference to Electron Microscopy," *Canad. J. publ. Hlth.*, 1948, 467–77; 17 refs., 6 figs.). Five methods are in use for the diagnosis of variola: (1) Isolation of virus by the inoculation of infected human material in rabbits with production of lesions. (2) Cultivation of virus on the chorio-allantoic membrane of the developing chick embryo. (3) The variola-vaccinia flocculation test. (4) Complement fixation. (5) Direct microscopic examination of films from scrapings of lesions for morphological evidence of elementary bodies. Attention is drawn to the use of the phase-contrast microscope for identification of elementary bodies and to the advantage of the electron microscope. The technique of preparing the exudate on formvar-covered 200-mesh electron microscope mounts is described.   G. M. F.

The Electron Microscopical Study of Cellulose Fibres.—W. G. KINSINGER and C. W. HOCK (" Electron Microscopical Studies of Natural Cellulose Fibres," *Ind. Eng. Chem.*, 1948, **40**, 1711–16). For metallic shadow-casting, fibres are beaten in water for 15–20 minutes in a Waring-Blender. Small drops of supernatant slurry are placed on a glass slide with a film of 0·3 p.c. parlodion in amyl acetate. After drying the specimen is shadowed with chromium or gold at an angle of 45° or higher. In making surface replicas, a film of 3 p.c. parlodion in amyl acetate is cast on a glass slide. Before the film is quite dry individual unbeaten fibres are pressed against it. The fibres are stripped off with tweezers and imprinted on the film. The film is then shadowed with chromium at an angle of 15°. Electron " stains " are mercuric chloride, lead acetate, silver nitrate, copper sulphate, uranyl acetate, thallium hydroxide, phosphotungstic acid, iodine and potassium iodide, and zinc chloride and iodide: these stains were applied as 1–20 p.c. aqueous solutions.   G. M. F.

Internal Structure of *Bacterium coli* by Electron Microscope Techniques.—J. HILLIER, S. MUDD, and A. G. SMITH (" Internal Structure and Nuclei in Cells of *Escherichia coli* as shown by Improved Electron Microscopic Techniques," *J. Bact.*, 1949, **57**, 319–38; 15 figs., 60 refs.). Growing bacteria on thin films of collodion overlying nutrient agar makes it possible to take electron micrographs of microcolonies that have not been subjected to mechanical disturbance or to alterations caused by suspending the cells in distilled water. The double objective lens with aperture greatly increases contrast and thus reveals internal structure of *Bacterium coli* more precisely than before. The nucleus is clearly shown and a three-dimensional latticework in the cytoplasm; the trabeculæ are aggregates of particles of the order of protein molecules. In intact cells the cell wall shows no internal structure, but in ghosts of phage-lysed cells the cell wall is divided into elliptical and circular segments.   G. M. F.

HISTOLOGICAL TECHNIQUE.

Metal Shadowing and Ordinary Microscopy.—D. B. SCOTT and R. W. G. WYCKOFF (" Metal Shadowing for the Optical Microscopy of Certain Tissues," *Amer. J. clin. Path.*, 1949, **19**, 63–6; 6 refs., 8 figs.). Metal shadowing can be successfully used on preparations for the optical as well as the electron microscope. It has been applied in the study of teeth, blood smears, skin, nails, and hair.   G. M. F.

Mass Preparation of Slides.—J. G. DACANAY (" A Method for Mass Preparation of Multiple Histopathological Slides," *Stain Technol.*, 1949, **24**, 99–102). Tissues from a single animal are placed together in 60-gauge cheese cloth, fixed in Zenker or formol-alcohol, washed, and brought up to 70 p.c. alcohol. Dehydration is carried out in *iso*propyl alcohol (70 to 99 p.c. for 7 hours), infiltrated, and embedded in paraffin with bayberry wax. Sections are brought down to water and stained with Harris's alum hæmatoxylin. G. M. F.

Differential Staining of the Anterior Hypophysis of the Rat.—B. BRISENO-CASTREJON and J. C. FINERTY (" An Azocarmine Stain for Differential Cell Analysis of the Rat Anterior Hypophysis," *Stain Technol.*, 1949, **24**, 103–7 ; 8 refs.). Tissues are fixed in Zenker-formol ; sections are brought down to water through Lugol's solution and sodium thiosulphate ; after staining with alum hæmatoxylin and washing with tap water and distilled water sections are placed in 80 p.c. alcohol for 3 minutes, aniline alcohol for 15 minutes, and then stained in azocarmine at 60° C. for 45 minutes. After rinsing in distilled water differentiation is carried out in aniline alcohol for 2–3 minutes, acid alcohol for ½–1 minute, and 5 p.c. phosphotungstic acid 1 hour. Sections are dehydrated in from 70 p.c. to absolute alcohol, counterstained in acid green solution for 5 minutes, xylene, and mounted in clarite. Alpha cell granules are purplish red ; beta cell granules light green, mitochondria orange-red ; red blood cells are brilliant orange. The Golgi apparatus shows up as a negative image in both alpha and beta cells. G. M. F.

Water-soluble Wax.—H. BLANK (" A Rapid Imbedding Technic for Histologic Sections employing a Water-soluble Wax," *J. invest. Dermat.*, 1949, **12**, 95–100). After fixation tissues are transferred direct to " Carbowax " (Carbowax, marketed by the Carbide and Carbon Chemical Corporation, is a mixture of polyethylene glycols: the most satisfactory embedding material is 1 part of Carbowax 1500, of the consistency of petrolatum, and 9 parts of Carbowax 4000, of the consistency of hard paraffin) in a paraffin oven at 54° C. The specimens are agitated from time to time and put through three changes of Carbowax, each for 1 hour. For the third and last change the tissues are transferred to Carbowax in a shallow container such as a petri dish. When the tissue has been in melted wax for 3 hours it is rapidly hardened by placing in an ice-box for a few minutes. Sections can be cut at from 2 to 10μ. All tissues can be thus treated, but specimens containing large amounts of gross fat are not adequately impregnated unless first placed in a fat solvent such as 1 part of Carbowax and 5 parts of acetone for 12 hours at room temperature. G. M. F.

Nigrosin for Protozoa.—R. M. ROSENBAUM (" Negative-staining of Protozoa with Nigrosin," *Stain Technol.*, 1949, **24**, 79–84 ; 11 refs.). One drop of animal-containing infusion is put on a slide and allowed to spread of its own accord. A drop of 10 p.c. aqueous nigrosin is then put on this drop and the whole is mixed and spread on the slide. To obtain a satisfactory thickness of the staining film the slide is put on its side and a piece of blotting-paper held against the edge. In this way excess stain is drained off. The slide is put on a warming table, dried, and mounted in clarite. No discoloration has been noted after 2 years. Ciliary bands appear black against the transparent cuticle; the nucleus is faint purple to purple black. Vacuoles and basal bodies are black. G. M. F.

Restaining Romanowsky Preparations.—E. HARTMAN (" Methylene Blue precipitated with Cadmium Chloride as a Restaining Agent for Blood and Tissue Protozoa," *J. Parasitol.*, 1948, **34**, Section 2, 37). Romanowsky-type stains fade very

easily: the blue colours are particularly unsatisfactory both from the standpoint of permanence and of restaining. A useful blue for restaining can be prepared by adding very slowly to 20 ml. of a 25 p.c. solution of cadmium chloride a solution of methylene blue (1 gm. in 40 ml. of distilled water). The resulting precipitate is collected on a filter-paper and dried. The weight of the recovered stain is in excess of 2·5 times the methylene blue used. One gm. of the precipitated stain is dissolved in 500 ml. of N/30 phosphate buffer at *p*H 7·4. This stock blue solution keeps indefinitely and can be used full strength, diluted, or combined with aqueous eosin. When combined with eosin a useful strength is 1 or 1·5 ml. 1 p.c. eosin and 30 ml. cadmium-methylene blue solution. Old slides must first be thoroughly cleaned of all oily and resinous materials: xylol followed by benzene is recommended. The staining time required varies with different specimens. When the film shows a slightly blue colour upon holding it up to the light the slide may be rinsed with tap water and dried. Plasmodia, trypanosomes, and toxoplasma may thus be restained. G. M. F.

Staining Cell Inclusions.—R. E. DUFFETT (" Hematoxylin-Neutral-red. A Stain for Cell Inclusions and Certain Tissue Elements," *Stain Technol.*, 1949, **24**, 73–8; 12 refs.) Hæmatoxylin is used as a nuclear stain followed by neutral red: the neutral red is differentiated in aniline-xylene and some virus inclusions and certain cell and tissue elements are thus brought into prominence. Sections are brought down to water and stained for 1 minute in Bullard's hæmatoxylin, though other hæmatoxylins will do. The differentiation and blueing are carried out in the usual manner: stain with a 1 p.c. solution of neutral red (Gurr, London, No. 244) in distilled water for 3 minutes. Rinse in water and differentiate in aniline-xylene, equal parts. Rinse in xylene to remove the aniline and mount in D.P.X. (polystyrene synthetic resin of Kirkpatrick and Lendrum, 1939). Balsam is not satisfactory as a mounting medium. The aniline-xylene differentiation is carried out by washing away the neutral red with water, blot with clean, dry filter-paper and flood with aniline-xylene until differentiated; usually 5–10 minutes is required for the nuclei to resume their original blue-back colour. Differentiation is stopped by rinsing in xylene. Tissues may be fixed in 10 p.c. formol saline or formol-sublimate. G. M. F.

Staining Ascosporogenous Yeasts.—E. E. EVANS, C. W. KING, and J. W. BARTHOLOMEW (" Differentiating Yeast Ascospores and Vegetative Cells," *Stain Technol.*, **24**, 85–6; 3 refs.). Ascosporogenous yeasts are stained with crystal violet, acid alcohol, and safranin. The spores are stained violet and the vegetative cells red. A smear of sporulating yeast was dried in air and then heat-fixed lightly. The slide is flooded with aniline crystal violet and heated for 3 minutes, replenishing the stain as it evaporates. The stain should give off steam, but not boil. (Crystal violet 5 gm., ethyl alcohol 10 ml., aniline 2 ml., distilled water 20 ml.) The slide is rinsed in tap water and destained for 15 seconds with 95 p.c. ethyl alcohol containing 3 p.c. hydrochloric acid. The slide is then rinsed with tap water and stained lightly with safranin (10–15 seconds) (2·5 p.c. safranin 0 in 95 p.c. alcohol, 10 ml., distilled 100 ml.). Rinse in tap water and blot dry. Spores are stained deep violet, as contrasted with light pink vegetative cells. Safranin should not be applied for too long or the vegetative cells are dark. G. M. F.

A Rapid Rosin-Celloidin-Paraffin Method.—E. D. CRABB (" A Rapid Rosin-Celloidin-Paraffin Method for embedding Tissues," *Stain Technol.*, 1949, **24**, 87–92; 6 refs.). Pieces of tissue are dehydrated in 95 p.c. alcohol, cleared, washed with ether and infiltrated with a solution containing parloidin 9·0 gm., camphor 3·0 gm., absolute

alcohol 200 ml., ether 200 ml., rosin 45·0 gm., and castor oil 10 drops. It requires 24–48 hours at room temperature, with occasional shaking, for the parloidin to dissolve, and another 5–24 hours for the rosin to go into solution. The following schedule is given for pieces of bone marrow: fluid is changed by decanting. Fix in 70 p.c. alcohol; pass through two changes of 95 p.c. alcohol for 5 and 10 minutes; then in carboxylol for 10 minutes; ether, three changes in 10 minutes; parloidin solution, 10 minutes to several days: evaporate to a firm gel or solid in 1–12 hours; chloroform vapour, 1–12 hours; trim and clear in carboxylol or anilin for 15 minutes; xylene, three changes in 15 minutes; infiltrate in paraffin (1–5 hours); embed and section with a rotary microtome. Tissue containing fat or oil should be defatted before being infiltrated with the parloidin or paraffin.   G. M. F.

Staining Tubercle Bacilli.—M. A. DARROW (" Staining the Tubercle Organism in Sputum Smears," *Stain Technol.*, 1948, **24**, 93–4; 2 refs.). Heating the staining fluid is not necessary to show up tubercle bacilli. A comparison was made of the Ziehl-Neelsen formula (0·3 p.c. basic fuchsin and 5 p.c. phenol in about 10 p.c. alcohol), the Kinyoun's solution (3·3 p.c. basic fuchsin, 8·0 p.c. solution of phenol), and a third formula intermediate in strength between these two. The Kinyoun's solution was as good in the cold as the Ziehl-Neelsen technique: staining was continued for 3–5 minutes and decolorization for 2 minutes in acid alcohol was sufficient.   G. M. F.

Hydrochloric Acid for Fixing Chromosomes.—D. U. GERSTEL (" Hydrochloric Acid as a Fixative for Root Time Chromosomes," *Stain Technol.*, 1949, **24**, 95–7; 4 figs., 6 refs.). After treating with 0·2–0·3 p.c. colchicine at 26° C. for 2 hours root tips are fixed and macerated in a 1 in 10 dilution of concentrated hydrochloric acid at 60° C. for 10–14 minutes. They are then washed, transferred to a staining dish with aceto-orcein or aceto-carmine or aceto-lacmoid for 10 minutes, immersed in a drop of the stain, covered, and squashed. The preparations may subsequently be made permanent by floating off the cover-slips in acetic alcohol, transferring cover and slide to absolute alcohol, and recombining them in diaphane (or cedarwood oil for aceto-lacmoid).   G. M. F.

Cytochemical Demonstration of Aldehydes.—J. F. DANIELLI (" A Critical Study of Techniques for the Cytochemical Demonstration of Aldehydes," *Quart. J. micr. Sci.*, 1949, **90**, 67–74; 4 refs., 1 fig.). For demonstrating aldehydes by the technique of Feulgen and Bersin the following technique is satisfactory. Pieces of tissue not more than 2 mm. thick are fixed in 8 p.c. neutral formaldehyde or in a solution with 8 p.c. formaldehyde and 5 p.c. acetic acid. After fixation for not less than 2 hours and not more than 5 days sections are cut on a freezing microtome. Sections are washed with distilled water and then allowed to stand in saturated mercuric chloride solution or cold N/10 hydrochloric acid for 15 minutes. The mercuric chloride or hydrochloric acid solution is sucked off and the sections are washed twice with distilled water. Sections are then allowed to stand in reduced fuchsin solution for 15 minutes. Fuchsin solution is sucked off and the sections washed with a solution containing SO_2. Three washes each of 5 minutes' duration are used. SO_2 solution is made by mixing 10 ml. of 10 p.c. sodium bisulphite solution with 10 ml. of normal hydrochloric acid, and diluting to 200 ml. Sections are washed with distilled water and mounted either in neutral balsam or glycerine jelly. Modifications of this technique allow for the differentiation of free aldehyde, aldehyde acetal, and aldehyde liberated by oxidation.   G. M. F.

The Feulgen Reaction.—CHONG-FU LI, M. STACEY and W. G. OVEREND (" The Feulgen Nucleal Reaction, *Nature, Lond.*, 1949, **163**, 538–40; 10 refs.). A discussion of the chemistry of the Feulgen reaction, which should be read by all those interested, leads to the conclusion that " in the hands of the cytologist the Feulgen reaction does locate the precise site of desoxyribonucleic acid."
G. M. F.

A Stain for Trichomonads.—R. B. GREENBLATT (" A Simple Stain for Trichomonads and Vaginal Epithelia," *J. med. Ass. Georgia*, 1949, **38**, 95–6; 1 fig., 2 refs.). Smears, dried in air, are fixed with equal parts of ether and 95 p.c. alcohol for 2 minutes or more. Without drying the slide is flooded with an 0·5 p.c. alcoholic solution of pinacyanole (a cyanine compound). Add a few ml. of a buffer solution of pH 4·0 (monobasic potassium phosphate 6·63 gm., dibasic sodium phosphate 2·56 gm., distilled water to 1 litre) for 10–15 seconds. Wash the slide in running water; dry by blotting; apply xylol for 2–10 minutes and mount in balsam. The trichomonads stain pink to lavender.
G. M. F.

Demonstration of Alkaline Phosphatase.—A. LOVELESS and J. F. DANIELLI (" A Dye Phosphate for the Histo- and Cytochemical Demonstration of Alkaline Phosphatase, with Some Observations on the Differential Behaviour of Nuclear and Extranuclear Enzymes," *Quart. J. micr. Sci.*, 1949, **90**, 57–66; 6 refs., 6 figs.). The method usually used for cytochemical localization of alkaline phosphatase depends on hydrolysis by the enzyme of β-glycerophosphate in the presence of calcium nitrate. Calcium phosphate is thus precipitated at the site of enzymic activity. An alternative method depends on enzymic hydrolysis of a phenol phosphate in the presence of a diazonium hydroxide. Both methods have disadvantages. A synthetic substrate, p-nitro-benzene-azo-4α-naphthol-phosphate, is employed as the sodium salt. Experiments with rat kidney indicate that traces of end-products are necessary before the enzyme can attack this substrate. If results with the new substrate are compared with those obtained with β-glycerophosphate it is found that some sites display more activity towards one substrate than to the other. The cytochemical localization of phosphatase with the new substrate is not as precise as with β-glycerophosphate.
G. M. F.

Detection of Iron.—H. BUNTING (" The Histochemical Detection of Iron in Tissues," *Stain Technol.*, 1949, **24**, 109–116; 14 refs.). The methods of fixing and staining iron in tissues and in demonstrating iron in hæmoglobin and nuclei are discussed. The best fixation is by Lillie's method—10 p.c. formalin buffered at neutrality (4 gm. monohydrated acid sodium phosphate and 6·5 gm. anhydrous disodium phosphate per litre) for 24 hours. The Prussian blue method is preferable to Turnbull's method. Equal parts of aqueous 2 p.c. sodium ferrocyanide and 2 p.c. hydrochloric acid are mixed immediately before use and filtered. Sections are placed in this fluid for 1 hour at room temperature; the fluid is changed after the first half hour. If a method is required that uses non-iron-containing reagents, Macallum's or Mallory's hæmatoxylin and Quincke's ammonium sulphide are useful. The section is placed in 10 p.c. aqueous ammonium sulphide for 1 hour at room temperature: the hæmatoxylin solution is prepared immediately before use by dissolving 5–10 mgm. of ether-washed hæmatoxylin crystals in a few ml. of absolute alcohol; to this is added twice the volume of boiled distilled water. Sections are stained for 1 or 2 hours: excess hæmatoxylin is removed by washing in equal parts of absolute alcohol and ether for 30 minutes and rinsing in distilled water. Mayer's paracarmine may be used for counterstaining. Sections are mounted in clarite and a pink filter on the light-source is an advantage.
G. M. F.

Golgi's Dichromate-Silver Method.

Golgi's Dichromate-Silver Method.—R. W. PORTER and H. A. DAVENPORT (" Golgi's Dichromate-Silver Method. I. Effects of Embedding. II. Experiments with Modifications," *Stain Technol.*, 1949, 24, 117–26; 9 refs., 13 figs.). Tissues are fixed for 48 hours at 25° C. in the following mixture, which is to be mixed in the order given, silver nitrate, 0·5 p.c. aqueous solution, 90 ml.; formalin, concentrated commercial, unneutralized, 10 ml.; pure pyridine, 0·05–0·1 ml. The *p*H should be 5·5–6·0; if too much precipitation occurs the addition of pyridine can be omitted. If specimens are fixed by perfusion, flush out the blood with plain 10 p.c. formalin and follow with the mixture. Slices are cut into blocks the thickness of 0·5–1·0 cm.: these slices remain in the fixative for 48 hours. The blocks are then rinsed in water and placed in 2·5 p.c. potassium dichromate (aqueous) to which 1 ml. of 1 p.c. osmic acid solution is added for each 100 ml. for 3–5 days. The tissues are then washed with water for 1 hour and in 50 p.c. alcohol for 1 hour to remove the dichromate solution. Dehydrate with 95 p.c. alcohol and absolute alcohol, remove alcohol with xylol, and embed in paraffin. Blocks may be allowed to remain in the dichromate solution for 1–2 weeks without harm and possibly with improvement.   G. M. F.

Block-staining with the Feulgen Technique.

Block-staining with the Feulgen Technique.—J. F. LHOTKA and H. A. DAVENPORT (" The Feulgen-Picric-Acid Block Stain; Additional Data," *Stain Technol.*, 1949, 24, 127–31; 4 refs.). Attempts to combine fixation and staining into a single step did not succeed with mammalian tissues, but gave fair results in frogs. Fixation in sublimate-acetic acid was unsatisfactory for block staining, and fixation in sulphosalicylic-picric acid gave poor Feulgen stains in sections. Soxhlet extraction of tissues with absolute alcohol removed Feulgen-positive, non-nuclear material with the exception of that in vascular endothelium, cartilaginous matrix, and elastic tissues. After such extraction the results are similar with both block and slide methods of staining. The regular section method is more specific for nuclei than the block method.   G. M. F.

Virus Inclusions and the Phase Microscope.

Virus Inclusions and the Phase Microscope.—J. J. ANGULO, O. W. RICHARDS, and A. L. ROQUE (" Demonstration of Viral Inclusion Bodies in Unstained Tissue Sections with the Aid of the Phase Microscope. I. The Inclusion Bodies of Yellow Fever, Herpes Simplex, Fowl Pox, and Distemper," *J. Bact.*, 1949, 57, 297–303; 21 refs., 10 figs.). Unstained sections from the above virus infections when viewed by the phase-contrast microscope show the presence of cytoplasmic and intranuclear inclusion bodies. The finding of Councilman lesions in the liver of a child who died of generalized herpetic infection adds one more to the conditions in which these lesions have been described (yellow fever, Rift Valley fever, fatal burns). For the demonstration of inclusion bodies unstained sections were mounted in paraffin oil and the cover-glass sealed with " gold size " to facilitate the use of an immersion objective.

G. M. F.

Dioxane Dehydration for Paraffin Embedding.

Dioxane Dehydration for Paraffin Embedding.—T. F. WALKER (" Dioxane Method of Dehydration of Tissues for Paraffin Embedding," *Amer. J. clin. Path.*, 1949, 19, 291–2; 1 fig.). Tissues are suspended in dioxane by means of threads. The water in the tissue is heavier than the dioxane and, as it diffuses out of the tissue, sinks to the bottom of the solution, where it is absorbed by calcium oxide. Tissues must not be over 5 mm. in thickness. At first the tissues are placed in dioxane 3 parts, water 1 part for 1 hour, and then in undiluted dioxane for 3–48 hours. Tissues are then transferred to Tissuemat (equal parts with melting points of 52–54° C. and 54–56° C.) for 8–15 hours   G. M. F.

Anæsthesia of Insects.—E. R. WILLIS and L. M. ROTH (" A Microscope Stage for Continuous Anæsthesia of Insects," *Science*, 1949, **109**, 230; 2 text-figs.). A stage is described for use with the dissecting microscope, having a circular cavity lined with a 60-mesh wire screen; under this a tube delivers carbon dioxide to secure continuous anæsthesia during experimental manipulation. F. C. G.

Mounting Small Insects.—M. L. BHATIA (" A Simple Medium for Mounting Small Insects," *Nature, Lond.*, **164**, 64). Canada balsam is unsatisfactory for mounting small insects and a medium of creosote and Canada balsam takes a long time to dry; thus preparations easily get distorted. A medium which can be prepared by dissolving pine rosin in eucalyptus oil gives good results. Its refractive index is 1·497, lower than that of Canada balsam. The medium acts as a clearing as well as mounting agent and dries hard in 3–4 days. Insects after 15–25 minutes in 95 p.c. alcohol can be mounted directly in this medium and they clear in about half an hour. The larvæ of mosquitoes should be pricked in the thoracic region while they are in the alcohol. G. M. F.

Fluorescent Microscopy and Acid-fast Bacilli.—H. E. LIND (" Limitations of Fluorescent Microscopy for Detection of Acid-fast Bacilli," *Amer. J. clin. Path.*, 1949, **19**, 72–5; 4 refs.).—Chemical treatment of sputum with substances other than phenol is disadvantageous and so is concentration by centrifugation. A dark room is essential to reduce interfering outside light and minimize the fatigue of the examiner. G. M. F.

CYTOLOGY.

Chromatin Threads.—W. G. P. LAMB (" Chromatin Threads from Cell Nuclei," *Nature, Lond.*, 1949, **164**, 109 ; 2 figs., 4 refs.). Thread-like bodies have been isolated from cell nuclei such as carp red cells and calf thymus. These bodies have been termed chromatin threads and sometimes isolated chromosomes. Nuclei were disintegrated by four methods and were examined by the phase-contrast microscope, the electron microscope, and with the ordinary microscope after fixation and staining. Evidence has been obtained that the threads are fragments of drawn-out nuclei. Thymocyte nuclei are interphase nuclei and it would not be expected that metaphase chromosomes would be obtained. G. M. F.

Protein in Golgi Apparatus.—ISIDORE GERSH (" A Protein Component of the Golgi Apparatus," *Arch. Path.*, 1949, **47**, 99–109; 5 figs.). The paper gives a description of what appears to be a carbohydrate-containing protein in the Golgi apparatus in the columnar cells of the mucosa in the duodenum of the rabbit and guinea-pig. Solvents and enzyme preparations were employed in an attempt to characterize this substance. Staining was by the method for polysaccharides described by Hotchkiss. This procedure involves the oxidizing to aldehydes of certain alcohol groups in carbohydrates bound to tissue substance and visualizing the compound so formed as a red colour by the use of the Feulgen reagent. When sections are coagulated with alcohol, the Golgi apparatus of the columnar duodenal cell appears as a delicate pink or red network. A supplement to the discussion, by Hotchkiss, is included on the specificity of the periodic acid–leukofuchsin method under the conditions described. Carbohydrates may occur free in sections of frozen-dried material or combined in the forms of polysaccharide (glycogen), with certain lipids, possibly lipoproteins, and glycoproteins.

In the comment the writer compares the results of the periodic acid–leukofuchsin method with the use of heavy metals. The reactions obtained by the technique employed are discussed in great detail and the author sums up in these words: " The Golgi apparatus may be a carbohydrate-protein complex, itself relatively inert, which possesses the peculiar submicroscopic structure that provides a suitable framework for the orderly arrangement of enzymes and other activities."   F. C. G.

Cytoplasmic Inclusions of the Spermatocyte of *Limnæa stagnalis*.—J. B. GATENBY and T. A. A. MOUSSA (" A Note on the Cytoplasmic Inclusions of the Spermatocyte of *Lymnæa stagnalis*," *La Cellule*, 1949, **52**, Fasc. 3, 297–308; 1 text-fig., 1 table, 1 pl.). In the pre-archoplasmic region of the Golgi apparatus of the spermatocyte of *Limnæa* there appear a number of granules which, on centrifuging, are shown to be the heaviest components of the cell and lie below the mitochondria. It seems that hitherto these granules have not been described, and the authors suggest that they may represent abortive protein yolk formation by the spermatocyte.

There also appears a group of closely packed granules near the Golgi material which is different from the above. None of these granules appears to stain vitally by neutral red or methylene blue, and it is pointed out that the statement made in 1925 by Madam Karpova that the dictyosomes do not stain vitally in methylene blue is correct. Neither with the ordinary light microscope nor the phase-contrast microscope have the authors been able to find precursor granules or vacuoles in which the neutral red or methylene blue droplets might be said to appear. Sudan black staining is discussed.   F. C. G.

ZOOLOGY.

Hydra and Gravity.—R. F. EWER (" The Behaviour of Hydra in Response to Gravity," *Proc. Zool. Soc.*, 1947, **117**, 207–18). Prior to the commencement of these investigations, it was casually noticed that, in a colony of *Hydra vulgaris* kept in a laboratory tank for routine class work, adult specimens changed their position but little, whereas young buds immediately after separation from the parent rapidly migrated up the side of the tank to the surface of the water. Now previous workers have not made any distinction between the behaviour of adults and buds, upward movement usually being considered aerotropic rather than geotropic.

A series of experiments was therefore carried out with a view to settling the question, these with few exceptions being carried out with newly separated buds in pond water at pH 8–8·4 and temperature 17°–22° C., oxygen concentration being air saturation. In view of *Hydra's* photopositive behaviour, all experiments were carried out in a black chamber, in which the direction, source, and intensity of the light could be carefully controlled. The species used in the experiments agreed fairly closely with the subspecies *attenuata*.

It was found that buds migrated upwards irrespective of oxygen concentration, behaving similarly whether this was high or low. A gradient of oxygen concentration also had a negative effect. It was deduced from these experiments that upward movement is a negative geotaxis. It is pointed out, however, that these experiments and their conclusions do not imply that *Hydra* has no reaction to oxygen concentration. Such a reaction may exist and might be shown in adults where the gravity reaction is weak, or by animals walking on a horizontal surface where no response to gravity could be made.

Investigation of the interaction of light and gravity showed that in specimens illuminated from the side the gravity reaction is four times stronger than the reaction

to the particular light intensity used, whereas when illuminated from below, reaction to light was greater than to gravity. It was thought that this latter phenomenon might be due to the direction of the light being unnatural, and that the animals might in time learn to ignore it and travel upwards when lit from below, but this was disproved by further experiment. This effect of bottom light is discussed at considerable length in terms of the known mechanism of the animal's reaction to light. Two explanations are examined, the first being that orienting movements are evoked by a decrease in light intensity, and these movements prohibit the movements involved in taking a step; and the second being that steps cannot be taken if the base of the animal is more highly stimulated by light than the oral end. Whichever of these possibilities is true, the effect of bottom light on an animal walking on a vertical plane will be the same, and its inhibitory effect on upward movement will be greater than would be expected simply on the basis of a klinokinetic orientation towards light. It would seem, therefore, that no new complexity in type of response or in neuromuscular organization need be postulated to account for this phenomenon.

For the investigation of the effect of pH on the gravity reaction, two sets of experiments were conducted. In the first the pH was lowered to 7·0 by bubbling carbon dioxide through the water. Further lowering to 6·0 was found to damage the animals. It was found that lowering of pH did not cause any significant increase in the strength of the gravity reaction of buds. Adults, which do not normally exhibit negative geotaxis, did so under the above conditions, showing that increase in carbon dioxide concentration, while not affecting the gravity reaction of buds, evokes negative geotaxis in adults. This reaction of adults made it possible to ascertain whether the response was to pH or specifically to carbon dioxide, steps being counted in normal pond-water (pH 8·4) and in water with pH lowered to 6·9 by running in 1 ml. of hydrochloric acid. The lowering of pH had no effect on the animals, the effect previously found with carbon dioxide being specifically due to this and not simply to pH change. Experiments also indicated that, while negative geotaxis can be evoked in adults by increasing the carbon dioxide concentration, lowering of oxygen concentration has no effect.

The effect of temperature was not fully investigated, but it seems that walking is most rapid at about 22° C. after which abnormalities appear. All walking ceased at 26°–27° C. The gravity response of buds lasts for at least 3 days after separation from the parent.

The author concludes that the gravity reaction described is of biological significance. That of buds acts as a method of distribution and prevents overcrowding. The response in adults evoked by carbon dioxide serves to bring them up to the surface when there is likely to be a shortage of oxygen lower down.   E. D. H.

ROTIFERA.

New Zealand Rotifera.—C. R. Russell (" Reference List of the Rotatoria of New Zealand with Ecological Notes," *Trans. R. Soc. New Zealand*, 1945, 75, 102–23). The list is prefaced by a short historical summary of studies on New Zealand Rotifera. The present author has gathered together and brought up to date the records of previous workers, and records twelve species not hitherto found in New Zealand. This list brings the known rotatorian fauna of the country to 156 species, embracing 51 genera. The author has found some fifty species in the vicinity of Christ Church during the three years preceding the publication of this list, and gives interesting data concerning the pH and thermal range of each species studied, together with other taxonomic notes.   E. D. H.

New Rotatoria from New Zealand.—C. R. Russell ("Additions to the Rotatoria of New Zealand. Part I," *Trans. R. Soc. New Zealand*, 1947, **76,** 103–408). Corrections to the Reference List noted above are here given, and fourteen additional species are listed for the first time, one of these being a new species of the Notemnatid genus *Rindia*, which the author has named *Lindia parrotti* sp. nov.

L. parrotti resembles *L. tecusa* Harring & Myers, superficially, but is considerably smaller and differs from the latter in being oviparous. The new species is annulated and similar to *L. truncata* Harring & Myers, although the annulations are much more developed, as in the genus *Taphrocampa*. A line drawing of the trophi is included, together with a rather indistinct photomicrograph showing the rotifer in lateral aspect carrying its egg. E. D. H.

REVIEWS.

The Laboratory Diagnosis of Honey-Bee Diseases.   Monographs of the Quekett
  Microscopical Club.—H. A. DADE, Williams and Norgate, Ltd., London, 1949.
  19 pp.   Price 2s.

This monograph of the Quekett Microscopical Club fills an important need in bee-
keeping literature, and collects in one publication a considerable amount of information
which could only previously be found in separate pamphlets or books.

The author quite correctly states that the bee pathologist should be a practical
beekeeper or closely associated with expert apiarists, as disease diagnosis is considerably
helped by observation of the state of the whole colony suspected.

In foul brood the author describes the drying up of diseased larvæ into scales, but
his description would here be aided by a photograph showing how to hold the comb
so that the scales can be seen.   Even a highly skilled beekeeper is liable to fail to spot
the scales at this stage of the disease.

The author's description and figures illustrating American foul brood are excellent,
and his instructions on making a bacterial smear will be most helpful to amateur
pathologists; a useful tip is given on a method of freeing the slide from grease.

All figures of bacteria and of spores are given careful measurements, which is a
most important aid for recognition.

The milk tests to distinguish between European foul brood and A.F.B. will be
new to many workers and will be an interesting help in diagnosis.   E.F.B. is fortunately
uncommon in most parts of the country.   Chalk brood is fairly common, particularly
in the West of England, not so much due to neglected hives, as the author suggests,
but rather to damp conditions—it is often found on Exmoor, for example.

Addled brood is rather common and is often alarming to beekeepers who mistake
it for a more serious complaint.   It may occur in larvæ of any age, sometimes those
only just hatched perish after a few hours of life.

Acarine disease is dealt with clearly and in full detail, as indeed it deserves to be,
the author stating that it is the most widespread and destructive of diseases of adult
bees.   This is quite correct, as many beekeepers know to their sorrow.

Many beekeepers' associations have honorary microscopists among their members
whose chief work is with this disease, and who deal with large numbers of specimens
each year.   Samples are best sent, however, without the food the author suggests.
If candy is sent with the bees they often become smothered in the sugary substance
and are then difficult to dissect properly.

There is an interesting note on optical equipment for diagnosis of acarine disease,
and the author is naturally in favour of the prismatic binocular.   This is, of course,
the best apparatus, but is unfortunately beyond the reach of the pockets of would-be
pathologists, and beekeepers up and down the country are quite successfully using
simpler dissecting microscopes.

The late Mr. Richard Beck, whose father founded the firm of R. and J. Beck, was

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honorary microscopist for the Somerset Beekeepers' Association and regularly used and recommended a simple dissecting microscope supplied before the war with lenses ×5, ×12, ×20 for a little over £5.

Mr. Beck also made a study of melanosis, and obtained many queens discarded by beekeepers in the county. He often found disease in the spermatheca, but was not able to establish the identity of the bacteria or moulds causing the trouble.

Nosema and amœbic infection are thoroughly and clearly dealt with, and the amateur microscopist reading this work will be grateful for the detailed instructions on the technique of examination for these diseases ; and the author is to be congratulated on this most thorough and helpful section. There should perhaps be some recognition of the work of Mr. Dennis of Middlesex on the subject of Malpighamœba.

Mr. Dade wisely does not spend much time on paralysis, a subject upon which much is often written but little known, and the microscopist may spend many weary and unfruitful hours in trying to find out the cause of this trouble.

The paragraph on disorders of the queen is most interesting, and the author states correctly that a queen often arrives by post to the microscopist with a request to explain her failure as a suitable mother to her colony.

Incidentally, Mr. L. E. Snelgrove, the author of several classic works on bee-keeping, is highly suspicious of imported queens, and has established the fact that nosema is often brought to this country by queens and their attendants imported from the Continent.

This monograph of 19 pages packed with valuable material, beautifully clear plates, and photographs will be welcomed by the many amateur microscopists in the bee-keepers' associations throughout the country.   R. J. P.

Discharge Lamps for Photography and Projection.—H. R. BOURNE. Chapman and Hall, 1948. 424 pages, 186 text-figs., numerous bibliographies and tables. Price 36s.

There has been need for an authoritative exposition of existing knowledge on discharge lamps; the book under review answers the need well. The reviewer has found in the book the answer to many technical difficulties connected with the use of discharge lamps. Special mention must be made of the excellent chapter on the development of the mercury discharge lamp ; this adequately covers the history and points the way to future developments ; it would be well to confine the use of the term fused silica to the translucent material used for laboratory ware and to use the term fused quartz for the transparent material used for optical apparatus, discharge lamps, and all cases where high light transmission is required. The information given by the author on such subjects as coated cathodes, electrical circuits for discharge lamps, composition of sealing glasses, with details of their thermal expansion and other properties, together with the excellent bibliographies make the book invaluable to designers and users of these lamps. The reviewer has detected only one serious error. The brief mention of the use of discharge lamps in ultra-violet microscopy refers in fact not to ultra-violet microscopy but to fluorescent microscopy; apart from this error the book gives a complete and accurate account of the subject of discharge lamps.   A. E. J. V.

Manual of the Polarizing Microscope.—A. F. HALLIMOND. Cooke, Troughton and Simms, Ltd. 107 pages, 49 figs. and text illustrations, bibliography.

This book, written by the mineralogist to the Geological Survey, is an authoritative account of the polarizing microscope and its accessories. The basis for the description

is the microscopes and equipment made by Cooke, Troughton and Simms, but the methods described are applicable to most good petrological instruments, the feature of note in the book is that it is the first description of how to use the newer kinds of petrological microscope which use polaroid as the polarizing medium in place of the nicol prism. Never before has so much practical detail, enforced by theory and experiment, been condensed into such a small space. It is difficult to single out some special part of a book of such general excellence for comment but the chapter on The Interference Figure may be cited as an example; it contains all that the practising petrologist or mineralogist needs to know on this subject.   A. E. J. V.

PROCEEDINGS OF THE SOCIETY.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, FEBRUARY 18TH, 1948, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the President.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society:

D. H. Browning.	Sittingbourne.
J. Bunyan.	London.
T. Keeling.	Wigston Fields.
G. W. Midgelow.	Manchester.
P. D. F. Murray.	London.
R. R. E. Perkins.	East Grinstead.
H. Polkinhorne.	London.
K. Powell.	London.
Joan H. Tabor.	North Harrow.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

G. Abdin.	London.
P. J. F. Croset.	Colchester.
F. W. Cuckow.	London.
C. Rudlin.	West Mersea.

Donations were reported from:

Buckton Collections—
 87 miscellaneous slides.

Mr. M. H. Mannering, F.R.M.S.—
 4 hæmatological slides.

Dr. C. Tierney, F.R.M.S.—
 508 mineralogical slides.

Mr. W. Williamson, F.R.S.E., F.L.S., F.R.M.S.—
14 Old French micro-slides in cases.
2 slide frames.

Paper.—The following communication was made:
Mr. Frank Baker, Rowett Research Institute—
" Microscopy in the Investigation of Starch and Cellulose Breakdown in the Digestive Tract."
A very cordial vote of thanks was extended to the speaker.

Announcement.—The President made the following announcements:
The Section for Industrial Microscopy will meet in the Hastings Hall on Wednesday, February 25th, 1948, at 6.00 p.m., when the following paper will be read—
Miss M. Dempsey, B.Sc., F.R.M.S.—
" The Structure of the Skin and Leather Manufacture."

The Biological Section will meet in the Hastings Hall on Wednesday, March 3rd, 1948, at 6.00 p.m., when the following paper will be read—
Mr. C. D. Ovey, B.Sc., F.G.S.—
" The History and Structure of the Foraminifera."

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, MARCH 17TH, 1948, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the President.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society:

G. Abdin.	London.
P. J. F. Croset.	Colchester.
F. W. Cuckow.	London.
C. Rudlin.	West Mersea.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

R. J. Beer.	Sutton.
M. M. Goil.	Bareilly, India.
E. Gurr.	London.
J. L. Kent.	Oxted.

N. Monkman.	Danger Island, N.S.W.
M. Morling.	Ipswich.
S. Morris.	Sutton.
F. E. J. Okenden.	London.
P. H. Sandwell.	Brentwood.
B. Savage.	Stourport.
J. C. C. Taylor.	Welwyn.
A. Walker.	Taunton.

Donations were reported from:

Mr. J. Reid—
4 photographs of Culpepper and Scarlet Microscope.

Mr. Noel Monkman—
1 photograph, 2 newspaper cuttings, 12 miscellaneous micro-slides, and 19 photomicrographs.

Mr. R. S. Clay—
6 antique specimen holders.

Admission to Honorary Fellowship.—Mr. J. E. Barnard was formally admitted to the Honorary Fellowship of the Society.

Papers.—The following communications were made:

Dr. R. J. Ludford—
" Application of the Barnard Ultra-Violet Light Technique."

Mr. J. Smiles, A.R.C.S.—
" New Development in Visual Light Microscopy."

Announcements.—The President made the following announcements:

The Section of Industrial Microscopy will meet in the Hastings Hall on Wednesday, March 24th, 1948, at 6.00 p.m., when the following paper will be read—

Mr. H. L. Shipp, B.Sc., A.R.I.C.—
" The Microscopy of Food."

The Biological Section will meet in the Hastings Hall on Wednesday, April 7th, 1948, at 6.00 p.m., when the following paper will be read—

Dr. F. Greenshields, B.Sc.—
" Stagnation—Relation to Microscopic Life."

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, APRIL 21ST, 1948, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

Minutes of the preceding Meeting were read, confirmed, and signed by the President.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society:

R. J. Beer.	Sutton.
M. M. Goil.	Bareilly, India.
E. Gurr.	London.
J. L. Kent.	Oxted.
N. Monkman.	Danger Island, N.S.W.
M. Morling.	Ipswich.
S. Morris.	Sutton.
F. E. J. Okenden.	London.
P. H. Sandwell.	Brentwood.
B. Savage.	Stourport.
J. C. C. Taylor.	Welwyn.
A. Walker.	Taunton.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

R. F. Bastow.	Chumleigh.
M. E. Haine.	Aldermaston.
E. P. Herlihy.	London.
S. L. Shaw.	Darlington.
M. M. Swann.	Cambridge.

Donations were reported from:

Dr. J. A. Murray, F.R.S.—
Royal Society Year Book, 1947–48; *Newton Tercentenary Celebrations* ; and *Analytical Microscopy*, by T. E. Wallis.

Papers.—The following communication was made:

Dr. E. S. Horning, M.A., Chester Beatty Research Institute—
" Researches on Cosmic Radiations at the High Alpine Scientific Station, Jungfraujoch." Followed by an illustrative film.

Dr. E. P. George, F.Inst.P., Birkbeck College—
" Physical Properties of Cosmic Radiation." Demonstration of cosmic showers.

A very cordial vote of thanks was extended to the Speakers.

Announcements.—The President made the following announcements:

The Section of Industrial Microscopy will meet in the Hastings Hall on Wednesday, 28th April, 1948, at 6.00 p.m., when the following paper will be read—

Dr. F. Smithson, F.R.M.S., British Pottery Research Association—
" Microscopy of Ceramic and Raw Materials."

The Biological Section will meet in the Hastings Hall on Wednesday, 5th May, 1948, at 6.00 p.m., when the following paper will be read—

    Miss Barbara Walshe—

       " Behaviour in Chironomus Larvæ."

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, 19TH MAY, 1948, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the President.

New Fellows.—The following candidates were balloted for and duly elected Ordinary Fellows of the Society:

R. F. Bastow.	Chumleigh.
M. E. Haine.	Aldermaston.
E. P. Herlihy.	London.
S. L. Shaw.	Darlington.
M. M. Swann.	Cambridge.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

P. A. Boot.	Glasgow.
J. D. Calloway.	Chester.
G. L. Fairs.	Widnes.
S. Ghobrial.	Khartoum.
S. W. Marston.	Crayford.

Balance Sheet.—The President called upon the Treasurer to submit the Audited Accounts for the year 1947. On the motion of Mr. C. C. Swatman, seconded by Mr. J. J. Jackson, the accounts were received and adopted.

A vote of thanks was passed unanimously for the work which the Honorary Auditors, Messrs. Hunter, Jones, Halford & Co., had done on behalf of the Society during the past year.

BALANCE SHEET AS

		£	s.	d.	£	s.	d.
Capital	LIABILITIES.						
Being	(a) Life compounded subscriptions from 1st January, 1877 to 31st December, 1947				2391	15	6
	(b) Quekett Memorial Fund				100	0	0
	(c) Mortimer Bequest				45	0	0
	(d) A. N. Disney Bequest				100	0	0
	(e) Amount received in respect of Sales of Books and Catalogues from the Library (surplus to the Society's requirements) to 31st December, 1947	343	19	0			
	Add Sales during 1947	6	19	0			
					350	18	0
	(f) Admission Fees to 31st December, 1946	753	18	0			
	Add Fees during 1947	111	6	0			
					865	4	0
	(g) A. Gandolfi Hornyold Bequest				100	0	0
	(h) F. Balfour-Browne—Gift				100	0	0
					4052	17	6
	Less Amount transferred to Superannuation Reserve in respect of year ended 31st December, 1935				100	0	0
					3952	17	6
Sundry Creditors, Specific Reserves and Accrued Charges—							
	Subscriptions paid in advance	38	18	4			
	Journal Subscriptions paid in advance	28	10	0			
	Accrued Charges	3	10	4			
	Reserve for expenses in connection with the Centenary Meeting	211	14	0			
	Reserve for Repairs to Instruments	100	0	0			
	Reserve for cost of printing Journal, Illustrating, Postages and Reprints	1250	0	0			
					1632	12	8
Rehabilitation Reserve—							
	Amount transferred from Income and Expenditure Account, subject to approval of Council				500	0	0
Income and Expenditure Account—							
	Excess of Income and Expenditure for year ended 31st December, 1947	486	10	8			
	Less Balance as at 31st December, 1946	55	11	6			
					430	19	2
					£6516	9	4

S. R. WYCHERLEY, *Hon. Treasurer.*

AT 31st DECEMBER, 1947.

ASSETS.	£	s.	d.	£	s.	d.
Furniture, Equipment and Instruments—						
As at 31st December, 1946	70	10	0			
Additions during the year	25	0	0			
	95	10	0			
Less Depreciation at 10% p.a.	9	11	0			
				85	19	0
Investments at Cost, less amount w/o				4415	10	3

£400 London and North Eastern Railway Co. 3% Debenture Stock.
£500 Nottingham Corporation 3% Irredeemable Debenture Stock.
£150 Metropolitan Water Board " B " Stock.
£612 London Midland and Scottish Railway Co. 4% Preference Stock.
£200 New South Wales 5$\frac{1}{8}$% Loan, 1947–57.
£100 3% Conversion Loan, 1948–53.
£421 1s. 3$\frac{1}{2}$% War Stock (Registered).
£400 3$\frac{1}{2}$% War Stock (Inscribed).
£300 Birmingham Corporation 3$\frac{1}{4}$% Redeemable Stock, 1957–62.
£400 3% Defence Bonds.
£250 3% Savings Bonds.
£600 3% Defence Bonds.
£1200 3% Savings Bonds.
Note.—The market value of the above investments at 31st December, 1947, was approximately £5463 10s.

	£	s.	d.	£	s.	d.
Sundry Debtors—						
Subscriptions and Journal Sales	213	5	6			
Less Reserve for Doubtful Debts	94	7	2			
				118	18	4
Cash at Bank and in Hand—						
National Provincial Bank, Limited—						
Deposit Account	500	0	0			
Current Account	1385	8	9			
In Hand	10	13	0			
				1896	1	9
				£6516	**9**	**4**

We have examined the Accounts and Balance Sheet with the Books and Vouchers of the Society, and having received all the information and explanations we have required, report that the above Balance Sheet is properly drawn up so as to exhibit a true and correct view of the Society's affairs at the 31st December, 1947, according to the best of our information, and the explanations given to us, and as shown by the books. We have verified the investments and Cash Balances.

(Signed) HUNTER, JONES, HALFORD & CO.,
Hon. Auditors.

45, *Ludgate Hill, London, E.C.4.*
5th *May,* 1948.

Dr.   INCOME AND EXPENDITURE ACCOUNT FOR

EXPENDITURE.	£	s.	d.	£	s.	d.
To Rent, Telephone and Insurance				172	4	1
„ Salaries				672	8	4
„ Journal Expenditure—						
Printing—provision						
Printing—actual	103	16	3			
Editing and Abstracting	13	16	6			
Illustrating	52	13	3			
Postages and Addressing	5	10	9			
Reprints	14	17	7			
				190	14	4
„ Other Expenditure—						
Library Books and Binding	15	7	9			
Stationery, Printing, Postages and Sundry						
Expenses	216	3	8			
Repairs and Renewals of Furniture	8	2	6			
				239	13	11
„ Depreciation on Furniture				9	11	0
„ Balance of Income over Expenditure				100	12	4
				£1385	4	0
„ Balance, carried down				502	6	0
				£502	6	0
„ Bad Debt Reserve, being subscriptions cancelled, due to resignations, war services, or lapse of Membership and previously brought into Income	326	14	2			
Less earlier Bad Debt Reserve not now required	310	18	10			
				15	15	4
„ Balance carried forward to Balance Sheet				486	10	8
				£502	6	0

THE YEAR ENDED 31st DECEMBER, 1947.   **Cr.**

	INCOME.	£	s.	d.	£	s.	d
By	Fellows Subscriptions	830	9	9			
„	Journal Subscriptions and Sales	370	5	4			
					1200	15	1
„	Journal Advertisements, less discount . . .				25	17	3
„	Income from Investments—						
	Gross amount	181	8	2			
	Less : Income Tax deducted at source . .	25	10	8			
					155	17	6
„	Bank Interest				2	9	11
„	Other Receipts					4	3
					£1385	4	0
„	Balance of Income over Expenditure, brought down				100	12	4
„	Donations				401	13	8
					£502	6	0
„	Balance, brought down				502	6	0
					£502	6	0

Donations were reported from :

Mr. C. F. Hill, M.Inst.MM., A.Inst.P., F.L.S., F.R.M.S.—
42 Lantern slides of Society's antique microscopes.
Sir Isaac Pitman and Sons, Ltd.
" Technical Optics," Vol. I.

Paper.—The following communication was given and discussed:

Mr. N. S. Macqueen, F.R.M.S.—
" The Work of Percy Smith." This was followed by an illustrative film.

A cordial vote of thanks was extended to the speaker.

Announcement.—The President made the following announcement:

The Section of Industrial Microscopy will meet in the Hastings Hall on Wednesday,
26th May, 1948, at 6.00 p.m.

The Proceedings then terminated.

A SOIRÉE

WAS HELD IN THE COMMON ROOM, HASTINGS HALL, OLD LIBRARY AND PILLAR ROOM
OF THE BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON, W.C.1,
ON WEDNESDAY, OCTOBER 6TH, 1948.

The following very kindly arranged demonstrations:

Professor W. T. Astbury, Dr. R. Reed, and Mr. A. Millard—
Electron Microscope Studies of Biological Structure.
Professor W. T. Astbury, Dr. R. Reed, Professor R. D. Passey, and Dr. L.
Dmochowski—
Ultracentrifugation and Electron Microscopic Studies of Tissues of Inbred
Strains of Mice.
Messrs. C. Baker, Ltd.—
Demonstration of the New Series 4, Inclined High-Power Binocular Micro-
scope.
Dr. R. Barer—
1. Results obtained with the Burch Reflecting Microscope.
2. A New " Joystick-control " Micro-manipulator.
3. Phase-contrast Photomicrographs.
Messrs. Cooke, Troughton and Simms, Ltd.—
Phase-contrast Microscopy.
The British Leather Manufacturers' Research Association—Miss Dempsey—
Variations in Fat Storage in Mammalian Skin.
Dr. I. M. Dawson—
Electron Micrographs of the Viruses of Vaccinia, Influenza, and Newcastle
Disease.

Dr. A. F. W. Hughes—
　Studies on Dividing Cells in Tissue Culture by the Phase-contrast Microscope.

Messrs. Measuring and Scientific Equipment, Ltd.—
　M.S.E. Microtomes and Comparison Microscope.

Dr. Conmar Robinson—
　Interferometer for studying Diffusion in High Polymers.

Dr. R. J. Ludford, Mr. J. Smiles, and Mr. F. V. Welch—
　Phase-contrast Photomicrographs and Ultra-violet Micrographs of Cells and
　　Micro-organisms.

Dr. Alex Stock—
　The Determination of Calcium in Histological Sections.

Mr. M. M. Swann—
　Recent Advances in Polarized Light Microscopy for Biological Purposes.

Curator of Scientific Collections, Mr. F. C. Grigg—
　Demonstration of Scientific Instruments and Microscopical Preparations
　　from the Society's Historical Collections.

The following films were shown in the Old Library:
　" Hydra." By the late Percy Smith. (1930.)
　" The Cultivation of Living Tissue." By the late Dr. R. G. Canti. (1925–30.)
　" Cell Division in Tissue Cultures."
　" Polymers."

Dr. A. F. W. Hughes and Dr. Conmar Robinson explained the scope of these
　films.

Light refreshments were served in the Common Room.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION
　HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, NOVEMBER 17TH,
　1948, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the
President.

New Fellows.—The following candidates were balloted for and duly elected
Ordinary Fellows of the Society:

P. A. Boot.	Glasgow.
J. D. Calloway.	Chester.
G. L. Fairs.	Widnes.
S. Ghobrial.	Khartoum.
S. Marston.	Crayford.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

Honorary Fellowship—
     Dr. J. A. Murray.   Elected 1919 Ordinary Fellow.

Ordinary Fellowship—

F. Baker.	Bucksburn.
C. J. Battison.	Leicester.
P. D. Chambers.	Sittingbourne.
M. G. L. Curties.	Sanderstead.
M. C. Das.	Edinburgh.
I. M. Dawson.	London.
G. Fearnley.	Montreal.
A. E. Halliwell.	Hampton Wick.
D. H. Haskell.	San Francisco.
H. Heywood.	Sidcup.
P. O. D. Hopps.	Durham City.
D. J. Kidd.	Croydon.
J. E. Marson.	Bradford.
W. W. McEwen.	Nottingham.
J. M. Naish.	Higher Denham.
H. D. Noronha.	Edinburgh.
H. G. Rogers.	London.
R. W. Scarff.	London.
F. R. Selbie.	London.
E. G. Smith.	Southern Rhodesia.
V. W. H. Towns.	London.

Donations were reported from:

Mr. A. C. Butterworth—
     Mechanical finger for mounting diatoms.

Lord Charnwood, F.R.M.S.—
     37 micro-slides—human eye, pathological.

The Institution of Civil Engineers—
     1 Beck microscope with accessories in mahogany case.

Messrs. Kodak, Ltd.—
     1 copy 16-mm. film—" Spermatogenesis in the Grasshopper."

M. Paul Chevalier—
     " Microscopie Practique," by G. Deflandre.

Dr. A. Pijper, F.R.M.S.—
     " Shape and Motility of Bacteria."   Abridged version.

Mr. James Smith, F.R.M.S.—
     Three guineas.

Mr. Ingram Hendey, F.R.M.S.—
     28 lantern slides of microscopes.

Dr. C. Tierney—
     Mahogany slide cabinet with 24 drawers.

Mrs. E. E. Merlin—
    The Merlin Collection of Microscopes and Accessories.

Messrs. Chapman and Hall—
    " Bacteriology." By F. W. Tanner and F. W. Tanner, Jnr.

Mr. J. W. Warburton—
    Three pounds ten shillings.

Messrs. Olliver and Boyd, Ltd.—
    " The Essentials of General Cytology," by R. A. R. Gresson.

Demonstration.—Dr. A. F. W. Hughes, Chairman of the Film Section, described the aims and scope of the newly instituted Film Library.
    The following selection of films was shown:

By Dr. A. F. W. Hughes of Cambridge—
    " Cells in Tissue Cultures."

By the late Dr. R. G. Canti—
    " The Cultivation of Living Tissue." Reel one.

By Dr. Comandon and M. de Fonbrune of the Pasteur Institut, Paris—
    " Cell Division of the Red Blood Corpuscles of the Newt."
    " Mitosis of Amœba Acanthamœba."
    " Myxomycetes."

A very cordial Vote of Thanks was accorded to the speaker.

Announcement.—The President made the following announcement:

The Biological Section will meet in the Hastings Hall on Wednesday, December 1st, 1948, at 6.00 p.m. when the following paper will be read:

Mr. R. Ross, M.A., F.R.M.S.—
    " The Diatom Genus *Rutilaria* Greville."

The Proceedings then terminated.

AN ORDINARY MEETING

OF THE SOCIETY WAS HELD IN THE HASTINGS HALL, BRITISH MEDICAL ASSOCIATION HOUSE, TAVISTOCK SQUARE, LONDON, W.C.1, ON WEDNESDAY, DECEMBER 15TH, 1948, AT 5.30 P.M., DR. R. J. LUDFORD, PRESIDENT, IN THE CHAIR.

The Minutes of the preceding Meeting were read, confirmed, and signed by the President.

New Fellows.—The following candidates were balloted for and duly elected Fellows of the Society:

Honorary Fellow—
    Dr. James A. Murray.   Elected Ordinary Fellow 1919.

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Ordinary Fellows—

F. Baker.	Bucksburn.
C. J. Battison.	Leicester.
P. D. Chambers.	Sittingbourne.
M. G. L. Curties.	Sanderstead.
M. C. Das.	Edinburgh.
I. M. Dawson.	London.
G. Fearnley.	Montreal.
A. E. Halliwell.	Hampton Wick.
D. H. Haskell.	San Francisco.
H. Heywood.	Sidcup.
P. O. D. Hopps.	Durham City.
D. J. Kidd.	Croydon.
J. E. Marson.	Bradford.
W. W. McEwen.	Nottingham.
J. M. Naish.	Higher Denham.
H. D. Noronha.	Edinburgh.
H. G. Rogers.	London.
R. W. Scarff.	London.
F. R. Selbie.	London.
E. G. Smith.	Southern Rhodesia.
V. W. H. Towns.	London.

Nomination Certificates in favour of the following candidates were read for the first time and directed to be suspended in the Rooms of the Society in the usual manner:

K. M. Greenland.	London.
H. W. Johnson.	Hayes.
H. F. Onley.	St. Albans.
J. H. Prince.	Morden.
B. Stanford.	London.
G. A. Whipple.	London.

Donations were reported from:

Messrs. Chapman and Hall—
  " Discharge Lamps," by H. K. Bourne.
Messrs. C. Baker—
  " West's Microscope," by Francis West.

Papers and Demonstrations.—The Merlin Collection of Microscopes was demonstrated and described by the Curator of Scientific Collections, Mr. F. C. Grigg, F.L.S., and the Keeper of Instruments, Brigadier H. G. Smith, C.B., O.B.E., M.C. The Curator Emeritus of Records, Dr. C. Tierney, spoke on the historical aspects of this collection.

The following communications were read and discussed:

Dr. P. E. Hughesdon—
  " The Use of Uranyl Nitrate for Permanent Metachromatic Staining."
Dr. Alexander Stock—
  " Determination of Calcium in Histological Sections."

A very cordial vote of thanks was accorded to the speakers.

Announcement.—The President made the following announcement:

The Biological Section will meet in the Hastings Hall on Wednesday, January 5th, 1949, at 6.00 p.m. when the following paper will be read—

Mr. N. Ingram Hendey, F.L.S.—

        " ' Reproduction Pattern ' as a Factor in Variation in Some Marine Diatoms."

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The Proceedings then terminated.

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INDEX OF AUTHORS.